Echocardiography Audit on Patients with Hereditary Haemochromatosis

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Echocardiography Audit on Patients with Hereditary Haemochromatosis

Lorna Doran

A dissertation submitted in partial fulfilment of the requirements for the School of Physics, Clinical and Optometric Sciences. Dublin Institute of Technology, Kevin Street for the degree of MPhil.

October 2016
I certify that this dissertation which I now submit for examination for the award of Master of Philosophy, is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the test of my work.

This dissertation was prepared according to the regulations for postgraduate study of the Dublin Institute of Technology and has not been submitted in whole or part for an award in any other Institute or University.

The work reported on in this dissertation conforms to the principles and requirements of the Institute’s guidelines for ethics in research.

Signed: ____________________________________________

Date: 28th October 2016
ABSTRACT

**Background:** The European Association for the Study of the Liver (EASL) Guidelines for Hereditary Haemochromatosis (HH) refer specifically to symptomatic Homozygous C282Y patients and provide an algorithm for treating such patients who are predisposed to iron overloading. The British Society of Echocardiography does not make provisions for HH patients per se. Cardiac failure is a known complication of severe iron overload although it is atypical.

**Aim:** To retrospectively investigate if an echocardiogram (echo) was warranted in the HH population at Louth County Hospital (LCH) Venesection Clinic based on the current guidelines.

**Methods:** A cohort of 833 HH patients was reviewed with respect to: Hyperferritinemia (HFE) genotype, echo results, serum ferritin levels, transferrin saturations, patient gender, age at HH diagnosis and co-morbidities.

**Results:** From the cohort investigated, 553 of 833 patients (66 percent) had echoes performed. When reviewing all echo results for these patients (553), 87 percent (480) recorded normal echoes. Consolidating all echoes performed where Serum Ferritin Level data was present (548) and then targeting patients with no co-morbidities only (345), the LCH Venesection Clinic data analysis demonstrates that 92 percent of this cohort’s echoes (319) show normal results.

**Conclusion:** The results proved the thesis hypothesis was valid and a recommendation that the number of patients receiving echoes for Hereditary Haemochromatosis should be reviewed and local policy changed to reflect a lower frequency rate for echoes required, thereby reducing the backlog waiting list.
Key words: Hereditary Haemochromatosis, Echocardiography, Cardiomyopathy, Venesection.

Phlebotomy
ACKNOWLEDGEMENTS

I would like to express my sincere thanks to my academic supervisor Professor Pat Goodman, and the doctors and staff at Louth County Hospital, Dundalk and Our Lady of Lourdes Hospital, Drogheda, especially Dr. Sengupta, Dr. Keohane, Dr. Brandon, Ms. Margaret Gray, Ms. Majella Joblin and Ms. Anna Caplice.
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<td>Two-dimensional</td>
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<tr>
<td>3D</td>
<td>Three – dimensional</td>
</tr>
<tr>
<td>A2C</td>
<td>Apical two chamber</td>
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<tr>
<td>A4C</td>
<td>Apical four chamber</td>
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<tr>
<td>ABC</td>
<td>Activity Based Costing</td>
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<tr>
<td>ACC</td>
<td>American College of Cardiology</td>
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<tr>
<td>AHA</td>
<td>American Heart Association</td>
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<tr>
<td>ALT</td>
<td>Alanine Aminotransferase (<em>ALT</em>) test is typically used to detect liver injury.</td>
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<tr>
<td>AST</td>
<td>Aspartate Aminotransferase (<em>AST</em>) is usually used to detect liver damage</td>
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<tr>
<td>ASE</td>
<td>American Society of Echocardiography</td>
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<tr>
<td>BSE</td>
<td>British Society of Echocardiography</td>
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<tr>
<td>C282Y</td>
<td>Tyrosine to replace Cysteine at amino acid position 282</td>
</tr>
<tr>
<td>CDC</td>
<td>Centres for Disease Control and Prevention</td>
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<td>DCM</td>
<td>Dilated cardiomyopathy</td>
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<td>DD</td>
<td>Diastolic dysfunction</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>DOX</td>
<td>Doxorubicin</td>
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<td>EAE</td>
<td>European Association of Echocardiography</td>
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<td>EASL</td>
<td>European Association for Study of Liver Disease</td>
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<tr>
<td>FRDA</td>
<td>Fredreich’s Ataxia</td>
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<td>H63D</td>
<td>Aspartic Acid to replace Histidine at Amino Acid position 63</td>
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<tr>
<td>HbA1c</td>
<td>Glycated haemoglobin</td>
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<tr>
<td>HEIRS</td>
<td>Haemochromatosis and Iron Overload Screening</td>
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<td>H &amp; I</td>
<td>Histocompatibility and Immunogenetics</td>
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<tr>
<td>HH</td>
<td>Hereditary Haemochromatosis</td>
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<tr>
<td>HFE</td>
<td>Hyperferritinemia</td>
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<td>HFE-HC</td>
<td>Hyperferritinemia Haemochromatosis</td>
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<tr>
<td>HJV</td>
<td>Hemojuvelin</td>
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<tr>
<td>HLAA-A*3</td>
<td>Human Leukocyte Antigen</td>
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<tr>
<td>HSE</td>
<td>Health Service Executive</td>
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<tr>
<td>HTN</td>
<td>Hypertension</td>
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<td>IOC</td>
<td>Iron Overload Cardiomyopathy</td>
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<tr>
<td>LA</td>
<td>Left Atrium</td>
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<tr>
<td>LCH</td>
<td>Louth County Hospital</td>
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<tr>
<td>LEAP-1</td>
<td>Liver-expressed Antimicrobial Peptide LFT</td>
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<tr>
<td>LFT</td>
<td>Liver Function Test</td>
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<tr>
<td>LV</td>
<td>Left Ventricle</td>
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<tr>
<td>LVH</td>
<td>Left Ventricular Hypertrophy</td>
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<td>LVEDD</td>
<td>Left Ventricular End Diastolic Diameter</td>
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<td>Abbreviations</td>
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<td>MHC</td>
<td>Major Histocompatibility Complex</td>
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<td>MRI</td>
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<td>MR</td>
<td>Magnetic Resonance</td>
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<td>MS</td>
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<td>MV</td>
<td>Mitral Valve</td>
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<td>NAFLD</td>
<td>Non-alcoholic Fatty Liver Disease</td>
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<td>RA</td>
<td>Right Atrium</td>
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<tr>
<td>RBC</td>
<td>Red Blood Cells</td>
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<tr>
<td>RV</td>
<td>Right Ventricle</td>
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<tr>
<td>SF</td>
<td>Serum Ferritin</td>
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<tr>
<td>SLC40A1</td>
<td>Solute Carrier Family 40 (Iron-Regulated Transporter)</td>
</tr>
<tr>
<td>Tfr2</td>
<td>Transferrin Receptor 2</td>
</tr>
<tr>
<td>T2*</td>
<td>Time 2* relaxation of the nuclei when radiofrequency fades</td>
</tr>
<tr>
<td>TS</td>
<td>Transferrin Saturation</td>
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<tr>
<td>TSat</td>
<td>Transferrin Saturation</td>
</tr>
<tr>
<td>TTE</td>
<td>Transthoracic Echocardiography</td>
</tr>
<tr>
<td>UK NEQAS</td>
<td>The United Kingdom National External Quality Assessment Service</td>
</tr>
<tr>
<td>US</td>
<td>Ultrasound</td>
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<td>USF2</td>
<td>Upstream Stimulatory Factor 2</td>
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Chapter 1: Introduction to an Echocardiography Audit on Patients with Hereditary Haemochromatosis

1.1 Introduction

The purpose of this study was to review the exponential increase in the number of echocardiograms (echoes) being requested for Hereditary Haemochromatosis (HH) patients from the Louth County Hospital (LCH) Venesection Clinic.

The demonstrated data trend of increased requests from the LCH venesection clinic is shown in Fig. 1.1. Taking 2009 as a baseline, 2014 showed a 1354% increase in venesection echoes completed. When related to the known capacity of the echocardiography department this increase in requests was creating a direct amplification in patient waiting lists. Most importantly, as requests are dealt with on a first come first served basis, the relative importance of patient treatment needs was not being taken into account. This could potentially lead to delay of urgent echoes critical to a patient treatment outcome.

![Number of Venesection Echoes Completed Annually](image)

Fig. 1.1 Trendline of Louth County Hospital Venesection Clinic Echoes Completed Annually
The objective of this study was to retrospectively investigate if an echo was warranted in the HH population of the LCH venesection clinic. Then, following from the conclusions of this study, recommend to the Gastroenterology department whether the guiding principles of LCH should be retained or revisited if contrary to the conclusions.

As a brief introduction to the clinical aspects of this thesis, the three central terms are defined and explained in this section.

An echocardiogram, as defined by the American Heart Association is a test that uses high frequency sound waves (ultrasound) to make pictures of the heart.

According to Pietrangelo’s definition (Pietrangelo; 2015), Haemochromatosis is:

“any genetic or acquired defect that directly or indirectly persistently causes unchecked transfer of iron into the blood (from the intestine and storage/recycling sites, such as spleen and liver) and toxicity in parenchymatous organs, can cause the clinical syndrome called hemochromatosis.”

Furthermore, “Hemochromatosis may be divided into primary (or genetic) hemochromatosis and secondary hemochromatosis” (Zakim and Boyers; 2011). Primary or classical HH is caused by defective genes inherited from the parent(s). It is associated with two mutations in the hyperferritinemia (HFE) gene namely; C282Y and H63D (see Fig. 1.2) named after the chromosome defect positions. This will be discussed further in chapter three. For the purpose of this audit, only primary HH is being reviewed.

“Hereditary hemochromatosis is an autosomal recessive disorder of iron metabolism. ‘Autosomal’ means that the gene is on one of the first 22 pairs of chromosomes, and not on the X or Y chromosome (HFE is located on chromosome 6). Therefore, males and females are equally affected by the disease. "Recessive" means that two copies of
the defective gene, one inherited from each parent, are necessary to have the condition. That means that you usually have to have two copies of the defective HFE gene before you are at risk of developing hereditary hemochromatosis.”

(www.hemochromatosisdna.com/dna-testing/about-the-dna-test)

If both parents are carriers

![Diagram showing probability of children with two parents as carriers](image)

(about 1 in 25 marriages), On average a quarter of the children will develop haemochromatosis, half will be carriers and a quarter will be normal.

If one parent has haemochromatosis and the other is a carrier

![Diagram showing probability of children with one parent as carrier](image)

(about 1 in 3,000 marriages) on average half of the children will develop haemochromatosis and the other half will be carriers.

If both parents suffer from haemochromatosis,

![Diagram showing probability of children with two parents as affected](image)

(a rare event, occurring in about 1 in 10,000 marriages) all the children will inherit two defective genes and all will have haemochromatosis.

Fig. 1.2 Haemochromatosis a recessive, inherited disorder.

Source: www.haemochromatosis-ir.com/how_is_haemochromatosis_inherited.html
Venesection (or phlebotomy) is the act of drawing or removing blood through a cut (incision) or puncture for the purpose of analysis, blood donations or treatment of blood disorders (Assi & Baz; 2014).

1.2 Thesis Structure

Chapter one introduces the thesis structure. This includes an introduction to the problem statement, purpose and objectives of the study, the author’s viewpoint as an Echocardiographer (a specially trained Cardiac Physiologist) and the reasons why this study was undertaken.

An outline of each chapter’s focus, its interaction with subsequent chapters and its relationship to the study is justified throughout the Introduction section. In addition, the study methodology is described and a brief summary of the conclusions is presented. Finally, potential future use for the study and the data is proposed.

Chapter two reviews the background of the study examining the guidelines put forward by the British Society of Echocardiography (BSE), the American College of Cardiology (ACC) and the European Association for the Study of the Liver (EASL).

Chapter three reviews HH. This is central to the study as the cohort of patient data analysed stem from the Haemochromatosis venesection clinic in LCH. Comments on HH definitions, its history and genetics are reviewed. Additionally, the prevalence and penetrance of HH in Europe and Ireland is assessed.
Chapter four focuses on a review of blood and its constituent parts in relation to HH. It is reviewed at human body system and cellular levels. Particular attention is paid to iron metabolism and iron homeostasis, Hepcidin (systemic iron regulatory hormone), the affects of Iron accumulation/overload and in particular cardiac manifestations and its relationship to HH.

Chapter five examines the human heart, its physiology and functions. The mechanisms of iron overload in the heart are examined and evidence of cardiological symptoms is considered. Next, transthoracic echocardiography (also known as diagnostic ultrasound, an echocardiogram, or an echo) is reviewed in general followed by particular assessment criteria for patients with HH. The American Heart Association (2015) explains an echocardiogram as a test that uses high frequency sound waves (ultrasound) to make moving pictures of your heart. Finally, systolic and diastolic dysfunction are examined and the usefulness of echo findings for HH treatment is reassessed.

Chapter six is a review of the research process and methodology the author employed during this study and the key data is given in evidence to support the conclusions.

Chapter seven is a presentation of this project’s audit data and analysis of the data. Fundamental measurements and indicators discussed in chapters three, four and five are reviewed and presented with figures and observations from the LCH venesection patient record data audit.
Chapter eight discusses the experiences, challenges and beneficial outcomes the study has engendered on the trend of HH Echo requests from the venesection clinic. It also reviews how this research may contribute to future research and learning in this field.

Chapter nine closes this study thesis. In this final chapter, the conclusions and three recommendations for the Gastroenterology department are presented for review.
Chapter 2: Background to the Need for a Primary Research Audit on Venesection Echocardiography Services

2.1 Introduction

In order to have a meaningful and unbiased review of the objective, an assessment of guidelines from the governing bodies of the BSE, EASL and ACC was completed.

The BSE guidelines are based on “literature searching conducted in Medline, EMBASE, Cochrane library for English-language meta-analyses and systematic reviews from 1990 – 2006” (Appendix 1) and provide recommendations for performing echoes based on clinical studies. The BSE does not make such provisions for HH patients per se. The indications do not mention haemochromatosis as an indication for Echocardiography. This does not preclude a physician ordering an echo should they discern that there is a clinical basis for the test. Intrinsically, HH alone with no other co-morbidities to support echo is not a BSE indication for echocardiography (see appendix 1 for further detail).

Contrary to the BSE recommendations, the EASL Guidelines for HH refer specifically to symptomatic homozygous C282Y patients only and provide an algorithm for treating such patients who are predisposed to iron overloading see Fig. 2.1. The algorithm addresses those patients with the potent version of HH (homozygous C282Y patients) only, but does not address those patients who are homozygous H63D; compound heterozygous C282Y/H63D; C282Y carriers nor H63D carriers. EASL Guidelines also note that glycated hemoglobin (HbA1c), serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) should be reviewed and if symptomatic, then echocardiography should be performed. AST, HbA1c and ALT are biochemical blood markers that indicate liver damage or hepatotoxicity.
The ACC also provides an algorithm for assessing patients who are ‘at risk’ of Iron Overload Cardiomyopathy (IOC), suggesting that an echo with full diastolic function assessment should be undertaken, see Fig. 2.2. The relationship of IOC with HH has been well considered in literature reviews (Schreiber, 1957). A fundamental point here is those ‘at risk’ of IOC need echocardiography. Conversely, it could be said that those not ‘at risk’ do not require echocardiography. This is a salient point this thesis will review.

Fig. 2.1 EASL Proposed algorithm for the diagnostic management of patients with C282Y homozygosity

Source: (www.jhep-elsevier)
Liu and Olivieri (1994) define IOC as “the presence of systolic or diastolic cardiac dysfunction secondary to increased deposition of iron in the heart independent of other concomitant processes.”

Fig. 2.2 Proposed clinical pathway to evaluate patients with idiopathic cardiomyopathy or those at risk for iron overload

Source: Adapted from PubMed Central, J Am Coll Cardiol. 2010 Sep 21; 56(13) 1001–1012. doi 10.1016/j.jacc.2010.03.083
These apparent contradictions between the clinical discipline guidelines regarding whether an Echo should or should not be performed on the venesection clinic patient cohort is a paramount question related to this thesis. The cost/benefit analysis of pursuing one path or the other from a positive clinical patient outcome, departmental capacity management and monetary viewpoint will be presented in the Discussion Chapter 8.

2.2 Author and Echocardiography Background

The author has more than 20 years of clinical cardiology experience in both the public and private sectors, is an accredited member of the BSE and has been employed as an Echocardiographer in LCH during the past ten years including the period of this study.

Echocardiography can be described as an ultrasound technique used to image the heart and will be discussed in detail specific to this thesis in Chapter 5. It provides diagnostic information about the heart's function and internal structure (Bonow et al., 2011).

As outlined previously, due to the increasing number of venesection clinic patients being referred for echo and the extended echo waiting list, the author decided to investigate whether echoes were warranted for the HH patient population.

In order to answer this, the clinical background behind why gastroenterologists made a request for echo contra to the BSE Guidelines as outlined earlier, had to be uncovered. Furthermore an understanding of why this gastroenterology problem was becoming a cardiology problem needed investigation.
Iron hypothesis of cardiovascular disease causes controversy. Aursulesei et al. (2014) recently suggested that this subject has been controversial for 30 years. They state many studies support iron’s role in cardiovascular disease whilst other studies found no evidence in iron as a risk factor. This paradox supports the purpose for this thesis and lends further confidence in the validity of the audit and the proposed secondary research.

2.3 Audit Background

Patient data relating to a cohort of 878 HH patients attending the LCH venesection clinic was audited to determine the validity of the echo requests.

The purpose of the venesection clinic is for phlebotomy or bloodletting to reduce the level of serum ferritin (storage iron) to a maintenance level. A serum ferritin maintenance level ensures that the patient’s iron levels remain within the expected normal range required for any individual. According to Davey et al. (1954) this treatment has been in existence since the 1940’s.

Iron levels in the blood are evaluated using biochemical markers i.e. serum ferritin, transferrin saturation and iron itself. Once the iron levels in the blood are reduced by phlebotomy to maintenance levels, the patients are no longer at risk of iron overloading and thereby not at risk of cardiomyopathy. This fact ties in with the ACC guideline referred to earlier.
Brandhagen et al. (2002) state that, “Life expectancy is usually normal if phlebotomy is initiated before the development of cirrhosis or diabetes mellitus.” Jacobs et al. in 2007 reiterate this stating that:

“Classical hereditary haemochromatosis (HH) is a disease relating to iron overload with an increase in physical symptoms over time, leading to organ failure and poor survival,”

and adds one salient point: that treatment of HH is very easy and requires removing excess iron by phlebotomy or bloodletting and thus “preventing the disease and increasing survival.” Hahalis et al. (2005) similarly state there is evidence that supports this and with sufficient medical therapy, IOC can be reversed when it is diagnosed prior to end stage heart failure.

To place this secondary research in context, it is important to depict how the relational elements regarding HH and the heart’s physiology were interlinked. Fig. 2.3 depicts a mind map of these relational elements.

![Fig. 2.3 Relational Elements of the Venesection Audit and the secondary research](image-url)
In addition to the secondary research, which gave a holistic view of the interrelations between venesection, HH, echo, blood, iron and heart physiology, a road map was envisaged and the audit actions were drawn up.

The audit actions roadmap consisted of a venesection patient database focusing on: age; gender; serum ferritin levels at diagnosis; serum ferritin levels at the time of echo; actual echo results of the patients and patient co-morbidity. The fundamentals of each category were examined so that a grasp of how all the interlinked elements related to the conclusion.

The next step was to gain Health Service Executive Ethics Committee approval to ensure that all research was obtained ethically and anonymously (see appendix 2). This was sought, and upon receipt of approval to proceed, the research data was collated. A database of 878 patients was created to allow the collection and analysis of the patient record data. The planned outcome of this research was to ensure that the LCH Guidelines could be reviewed and improved with this evidence based data.

The outcome of this retrospective patient record audit would be to design a protocol for selecting or deselecting patients for echo who have HH. The main purpose of the project was to investigate and gather data, in the form of a patient record audit, of the list of patients attending the venesection clinic at LCH.

From a customer (patient/doctor/HSE) viewpoint, this research has redefined hospital protocol ensuring that patients, who required echoes, received them in a timely manner and that appropriate resources were applied, cost effectively, resulting in achievement of efficient patient and clinical outcomes.
In conclusion, this dissertation was written from a learning outcome/research perspective and has retraced the steps that were necessary to understand HH, how it affects the physiology and myocardium of the heart and in turn what echo results would be expected and what echo results were actually reported. The data obtained was retrospective and spanned a number of years. This dissertation will also discuss the outcomes of the research and suggest some recommendations as a result of the data obtained.

2.4 Chapter Summary

Chapter two has given an overview of the reasons for the decision to pursue an audit of patient records of the HH population attending the LCH Venesection Clinic and sought to gain an understanding of why these patients were reviewed for echo.

Literature research was undertaken to identify the various facets of the disease and of expected clinical outcomes anticipated from echocardiography. This literature research helped to structure the thesis and give a full and balanced view of both a gastroenterology and cardiology in relation to the very prevalent Northern European and Irish disease that is HH.

In the next chapter Haemochromatosis was defined and reviewed in terms of its history, its penetrance, and prevalence in populations and hones in on its most common form: Type I Hereditary Haemochromatosis (or classical HH). Type I HH is this most prevalent haemochromatosis in Ireland and this thesis main focus.
CHAPTER 3 : HEREDITARY HAEMOCHROMATOSIS – CURSE OF THE CELTS!

3.1 Introduction

Hereditary Haemochromatosis is the most common genetic disorder among Caucasians, predominantly those of Northern Europe with Celtic or Nordic ancestry, “with a prevalence of approximately 1 per 220-250 individuals” (Adams et al.; 2005; Kowdley, 1999). Feder et al. (1996) suggest carrier frequency is estimated at one in ten individuals of Northern European extraction. In Ireland carrier frequency is one in five (Nicholson; 2013). Nicholson (2013) states that, “over 93% of Irish HH patients are homozygous for the HFE gene C282Y.”

“Hemochromatosis may be divided into primary (or genetic) hemochromatosis and secondary hemochromatosis” (Zakim and Boyers, 2011). Primary or classical HH is associated with two mutations in the HFE gene namely; C282Y and H63D as explained in chapter one.

To fully understand HH, a review of Haemochromatosis literature commenting on a range of definitions and the genetic mutations causing this disorder is required.

To appreciate the complexity of this disease, blood and its relationship with iron distribution and hepcidin, which regulates iron homeostasis, is reviewed in chapter four. While in chapter five cardiac and more specifically echocardiographic manifestations of HH and how both of these subjects relate to one another in the treatment of HH patients is reviewed. The logical approach of these secondary research topics is demonstrated below in Fig 3.1.
Fig. 3.4 An overview of literature review topics and their relationship with this study

3.2 Hereditary Haemochromatosis Defined

The Centres for Disease Control and Prevention (www.cdc.gov) state succinctly that “Haemochromatosis is about iron.”

However, from a research and clinical perspective a range of definitions from eminent scholars must be reviewed in order to fully appreciate the complexity and simplicity of Haemochromatosis.
A world leading expert on Haemochromatosis, Pietrangelo, defined it in 2006 as follows:

“Hemochromatosis or Hereditary Hemochromatosis should be defined today as a hereditary iron loading disorder, multi genic in nature, caused by a genetically determined failure to prevent unneeded iron from entering the circulatory pool and characterized by progressive parenchymal iron overload with potential for multi organ damage and disease.”

Pietrangelo (2010) shortened this definition of Haemochromatosis to “a well-defined syndrome characterized by normal iron-driven erythropoiesis and the toxic accumulation of iron in the parenchymal cells of the liver, heart and endocrine glands.”

Pietrangelo (2015) also goes on to mention that it is recognised that haemochromatosis can be attributed to partial or total loss of the activity of hepcidin, a small cysteine-rich antimicrobial peptide hormone synthesized by the liver. This hormone controls iron entry into the bloodstream. Hepcidin is discussed more thoroughly in chapter four.

Allen et al., (2008) go on to define HH as an inherited condition of dysregulated iron absorption that can lead to total-body iron overload with secondary tissue damage in a wide range of organs and that iron overload related disease in patients with HH is characterised by “the presence of cirrhosis, severe fibrosis, hepatocellular carcinoma, or arthropathy and C282Y homozygosity.”

The Powell et al., (2005) definition is similar stating HH “is a primary inherited disorder of iron metabolism leading to progressive iron loading of parenchymal cells of the liver and
other organs with diverse clinical manifestations, including cirrhosis, diabetes and skin pigmentation.”

Britton et al., (2002) repeats the same stating that “Hereditary hemochromatosis (HH) comprises several inherited disorders of iron homeostasis characterized by increased gastrointestinal iron absorption and secondary tissue iron deposition.” Andrews (2008) expands on this definition and states that the “dysregulated intestinal iron absorption is mediated at the level of the duodenal enterocyte.”

It is quite clear that there is little variation and no substantive dispute in the definition of HH by the leading experts in the field. Comparing these definitions, a number of salient points are common and all definitions describe a disease process; namely iron overloading or toxic accumulation and possibly organ damage.

Lauffer (1991) describes Haemochromatosis as a “disorder of iron metabolism... described as one of modern medicine’s biggest oversights.” An oversight because there is ambiguity around the symptoms and patients are often misdiagnosed because the symptoms are common and non-specific. On the other hand, Griffiths (2011) suggests a low index of clinical suspicion is advisable to avoid delays in diagnosis which could lead to iron overload and ultimately organ damage.

Although these are not the conventional definitions of Haemochromatosis, the literature points to possible controversy over not only its diagnosis but also over the risk of developing the disease and furthermore over the prevalence of the higher end risks of the disease. Beutler et al., (2002) state that the “fully expressed disease with end-organ manifestations is
seen in fewer than 10% of these individuals.” This ambiguity also spills into the management of the disease and hence the question posed initially in this thesis: is an echo warranted in the HH population at LCH venesection clinic based on the current guidelines or is there a need for change?

So, now a firm understanding of what HH is and its possible effects on this group of individuals has been established. The definitions provided above are all relatively recent in comparison to the history of Haemochromatosis which has its origins going back thousands of years. The next element geneticists are interested in is where the mutation came from.

### 3.3 A Brief History of Hereditary Haemochromatosis

Taking a look back chronologically, Haemochromatosis has its origin founded in the Neolithic or New stone age: “the Northern European hunter gatherer diet with traditional iron-rich high dependence on mammals and/or shellfish shifted to heavy dependence on grains and milk products in the Neolithic” (Straus 1977; Cordain et al., 2000; 2002). McCullough et al. (2015) hypotheses about the fact that “positive selection for Hyperferritinemia (HFE) began during or after the European Neolithic with the adoption of an iron-deficient high grain and dairying diet and consequent anemia, a finding confirmed in Neolithic and later European skeletons.”

Crowe (2009) states that researchers from the Mater Hospital say it happened 50 generations ago, about 900 AD. Raha-Chowdhury and Gruen (2000) contradict this theory and suggest that the HFE gene as found has “23 mutations in 730 meioses, suggesting an age for the
original C282Y mutation of approximately 200-250 generations earlier, or roughly 6,000+ years ago, assuming 30 years per generation.”

Despite these contradictory opinions from approximately 5000-4000 BC to 150 years ago, this Neolithic condition found its way through time and Haemochromatosis was first described by Dr. Armand Trosseau (1885), a French physician, as “cirrhose portale avec diabete bronze” or portal cirrhosis, diabetes mellitus and bronze skin pigmentation or ‘bronze diabetes.’ The actual naming of Haemochromatosis is attributed to a German scientist, Von Recklinghaussen (1889) who named it from: "hemo" for blood and "chroma" for color, referring to the characteristic bronze skin pigmentation from iron overload.

Sheldon, (1935) recognised that Haemochromatosis was caused by excess iron deposition and that it was hereditary. Then in the 1970’s Simon et al. described the genetics of haemochromatosis autosomal recessive disorder of the short arm of chromosome 6 encoding the human leukocyte antigen (HLA-A*3) also known as (HLA)-A3 complex. The World Health Organisation Nomenclature Committee for Factors of the HLA system renamed HLA as HFE (hyperferritinaemia), (Bodmer et al., 1997).

The Hyperferritinaemia (HFE) gene or ‘haemochromatosis gene’ was identified by Feder and his colleagues in 1996. Zhou et al., (1998) demonstrated proof of the HFE gene when “knockout of the mouse HFE gene resulted in iron overload.” These breakthroughs sparked a new era of discovery and in 2001 the hormone hepcidin was noted to be deficient in HH patients. This hormone is essential for the control of the body’s iron balance. In its absence HFE or iron overload results. Hepcidin will be discussed in further detail in chapter four.
3.4 Genotyping Haemochromatosis

Earlier it was mentioned that various mutations exist in relation to Hereditary Haemochromatosis. The two most prevalent mutant alleles associated with haemochromatosis are the C282Y and H63D variants (Merryweather et al., 2000).

However, classification of Haemochromatosis is dependent on a number of different factors: genetic profile, method of inheritance and onset age. Confusion can occur when describing this condition as there are a number of generic names, for example: Classic HH; Haemochromatosis Type 1; Major HH; or minor HH. A distinction also needs to be made because Haemochromatosis can be primary HFE, primary non–HFE or secondary Haemochromatosis.

There are also four types of Haemochromatosis and associated genes as listed in Table 3.1. For the purposes of this thesis, Type I is being considered primarily as it is the most common form. It is known as Classic HH and can be defined as “an autosomal recessive iron-overload disorder associated with mutation of the HFE gene, which is located on chromosome 6” (Feder et al., 1996).

Primary Hereditary Haemochromatosis (Type 1) is associated with a gene defect that is inherited. Secondary Haemochromatosis or secondary iron overload is not inherited and the cause of iron deposition is due to another disease. Secondary Iron overload is caused by iron-loading anaemias, parenteral iron overload, chronic liver disease and miscellaneous causes (Nicholson, 2013). Juvenile hemochromatosis is caused by a mutation in the HJV gene. Neonatal hemochromatosis is an autoimmune disease. See appendix 3 for an expanded list of Classification of Iron Overload Syndromes.
<table>
<thead>
<tr>
<th>Type of Primary Haemochromatosis</th>
<th>Common Name</th>
<th>Associated gene and gene product</th>
<th>HFE/Non-HFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>Classic haemochromatosis</td>
<td>HFE (HFE)</td>
<td>HFE related</td>
</tr>
<tr>
<td>Type 2A</td>
<td>Juvenile haemochromatosis</td>
<td>HFE2 (hemojuvelin or HJV)</td>
<td>Non-HFE related</td>
</tr>
<tr>
<td>Type 2B</td>
<td>Juvenile haemochromatosis</td>
<td>HAMP (hepcidin)</td>
<td>Non-HFE related</td>
</tr>
<tr>
<td>Type 3</td>
<td>Tfr2-related haemochromatosis</td>
<td>Tfr2 (transferrin receptor–2)</td>
<td>Non-HFE related</td>
</tr>
<tr>
<td>Type 4</td>
<td>Ferroportin-related iron overload</td>
<td>SLC40A1 (ferroportin)</td>
<td>Non-HFE related</td>
</tr>
</tbody>
</table>

**Table 3.1 Classification of iron overload syndromes.**

**Source:** Adapted from Fig. 3 Hand Book of Liver Disease, Dand and Kowdley chapter 16

Fig. 3.2 adapted from (Jacobs *et al.*, 2007) graphically demonstrates the five stages of HFE related HH (Type 1) together with the various strategies for screening HH. It illustrates the effects of elevated serum iron parameters i.e. elevated serum transferrin saturation (TS) and serum ferritin (SF) and its prevalence within the population. Beginning at stage 0 where there are no symptoms, biochemical nor clinical. Over time this migrates to increases in transferrin saturation, then adds increases in serum ferritin with no clinical symptoms but then moves to the more serious clinical symptoms affecting quality of life and then finally onto stage IV where clinical symptoms can manifest in organ damage and early mortality.
Accompanying these biochemical and clinical symptoms we have general population screening, family screening where a family member with C282Y has been found to carry the gene, and then targeted screening. Screening will be touched on later.

**Fig. 3.5 Five Stages of the HFE related Hereditary Haemochromatosis**

**Source:** Adapted from Jacobs *et al.*, 2007.

There are a number of significant points in relation to HH that makes an individual more susceptible to iron overload. As a result these iron overload individuals are at an increased risk of a cardiac manifestation. These cardiac manifestations present usually after the fourth decade when iron overload becomes apparent in an undiagnosed individual. This accumulation takes years as a person with HH absorbs only a small amount of iron each day in excess of the body’s needs. The body normally stores about 4 g of iron.
The real iron deposition occurs after 40 years of accumulation when the body iron stores have reached 15 g to 40 g see Fig 3.3. This is investigated more fully in chapter four.

This storage occurs “in parenchymal cells of the liver, pancreas and heart liable to result in organ damage. The clinical onset usually appears after midlife, and the clinical outcomes and severity of the disease varies considerably.” (Niederau *et al.*, 1999). It is worth noting that “Men with type 1 or type 4 hemochromatosis typically develop symptoms between the ages of 40 and 60, and women usually develop symptoms after menopause,” (US National Library of Medicine, 2016).

![Iron Stores and Hereditary Hemochromatosis](image)

**Age in years**

**Fig. 3.6 Iron Stores and Hereditary Hemochromatosis: Relationship between total body iron stores and clinical manifestations of HH over time.**

**Source:** Brandhagen, D., Fairbanks, V., Baldus, W., (2002)
Three longitudinal population screening studies in which patients were followed for more than 20 years have shown that disease progresses in only a minority of untreated patients with HFE C282Y (Olynyk et al., 2004; Andersen et al., 2004; Allen et al., 2008). Some subjects with compound heterozygosity (H63D/C282Y) or H63D homozygosity (H63D/H63D) also present with abnormal iron parameters, or even increased deposits of hepatic iron, but these patients usually have disease cofactors (Gurrin et al., 2009; Ramakrishna et al., 2013).

The cardiac manifestations of iron overload include diastolic dysfunction that can progress to Cardiomyopathy. These shall be discussed in chapter five in more detail.

The various genes related to haemochromatosis types and secondary iron overload have been discussed. However, focus must be drawn to the fact that to be diagnosed with HH, the patient must have not only a genotype but also a phenotype (physical characteristic). This is graphically represented in Fig. 3.4.

Fig. 3.7 Genotype and Phenotype.

Source: Adapted from desktop article Fletcher and Halliday (2002)
Fig. 3.4 illustrates a generic mechanism through which patient diagnosis can be made. When a patient presents with abnormal iron studies or symptoms of HH, blood tests are used to assess the biochemical markers of iron overload, namely: transferrin saturation (TSat), serum ferritin (SF) and liver function tests (LFT). If these tests demonstrate the TSat marker is greater than 45%, genetic screening blood tests are used to confirm the existence of the C282Y or H63D mutation(s). The result could be homozygous, heterozygous or the patient could be a carrier. In the case of the LCH venesection clinic patients, they have blood tests and genetic screening requested by their General Practitioner service or the hospital inpatient or outpatient’s service.

The symptoms and biochemical markers described in Fig 3.4 are considered to be the physical characteristics or phenotype of the disease. In order to be diagnosed with HH both the phenotype and genotype must be present.

The genotype refers to the genetic makeup. Genes are carried within chromosomes. In humans, each cell has 46 chromosomes. These are divided into 23 pairs, one copy from each parent. As Feder et al., (1996) state, “Classic hereditary hemochromatosis is an autosomal recessive iron-overload disorder associated with mutation of the HFE gene, which is located on chromosome 6.” Autosomal recessive means that the presence of either gene (C282Y or H63D) is required from both parents to have the chance of a child developing the HH disease. So at “conception each child of parents who are both carriers have a 1 in 4 (25%) chance of being an unaffected non-carrier; a 2 in 4 (50%) chance of being a carrier and a 1 in 4 (25%) chance of inheriting the condition,” based on the Hardy-Weinberg Equation (www.geneticseducation.nhs.uk). Fig. 3.5 illustrates family screening and the possible genetic outcomes previously described.
Fig. 3.8 Family Screening and Possible Genetic Outcomes

Source: Adapted from Haemochromatosis diagnosis, John Hopkins https://gi.jhsps.org/

In molecular genetic analysis terms, this will be described as a patient being either homozygous for the C282Y mutation, written as C282Y/C282Y in the Hyperferritinemia (HFE) gene or H63D/H63D (homozygous for H63D mutation) in the HFE gene.

Compound heterozygotes written as C282Y/H63D have one HFE gene mutation from one parent and one from the other. Patients can also be carriers having only one copy of the HFE gene mutation. The carrier molecular genetic analysis result would be written as C282Y/n or H63D/n depending on which HFE mutation is carried. HH is predominantly associated with
the C282Y mutation and to a lesser extent H63D mutation. The HFE gene will now be focused on. “The hemochromatosis gene, HFE, is located on chromosome 6 in close proximity to the HLA-A locus,” (Bittencourt et al., 2002). Merryweather et al. (1997) describe the two mutations C282Y and H63D. The HFE gene encodes a 343 amino acid complex class 1 type molecule. Feder et al., (1996) clarify this further by describing the HFE gene defect and is a “G - to –A missense mutation leading to a substitution of tyrosine for cysteine at amino acid position 282 of the protein product (C282Y)” at nucleotide 845 of the HFE gene (Fig. 3.6) or a mutation leading to a substitution of ‘histidine at amino acid position 63 (H63D)’ at nucleotide 187 of the HFE gene (Fig. 3.7).

![Diagram of C282Y mutation](https://www.jhmcall.org/GDL_Disease)

**Fig. 3.9 Major Hyperferritinemia (HFE) associated polymorphism C282Y:**

Chromosome 6, the HLA-A gene and the locus of the C282Y mutation

**Source:** https://www.jhmcall.org/GDL_Disease.
Fig. 3.10 Minor Hyperferritinemia (HFE) associated polymorphism H63D: Chromosome 6, the HLA-A gene and the locus of the H63D mutation

Source: https://www.jhmicall.org/GDL_Disease.

3.5 Prevalence in the general Irish population

Prevalence is the percentage of a population that is affected with a particular disease at a given time (www.merriam-webster.com).
HH is the most prevalent inherited disease in Ireland and in fact Ireland has the highest reported prevalence of HH in the world (Ryan and Crowe, 2010). According to Crowe (1998) over 93% of Irish HH patients are homozygous for the HFE gene C282Y mutation. Crowe (2009) from the Mater's Liver Unit says the spread of haemochromatosis "around the world is associated with the Irish Diaspora." Nicholson (2013) describes haemochromatosis as “an autosomal recessive disorder of iron metabolism, and the most common genetic disease in Europe.” Limdi and Crampton (2004) add that “it affects the Caucasian population with a prevalence of between 1 in 200 and 1 in 500 with an even higher prevalence likely in the Irish population.” More recent data by Bacon et al. (2011) state that HH occurs in populations of northern European origin with Celtic or Nordic ancestry; with a prevalence of 1 per 220–250 individuals.

The subject of HH has been examined by researchers throughout the world and a more comprehensive a study could not be found than a recent publication from the European Association for the study of Liver Disease (EASL) in the Journal of Hepatology.

Using a meta-analysis of 36 studies, examining 127,613 individuals from 22 countries the EASL Clinical Practice Guidelines highlight the prevalence of the common HFE polymorphism in the general population as shown in Appendix 4. In this study the major HFE polymorphism C282Y, was found in 1 in 260 individuals or 6.2% (in the pooled cohort of 127,613) whilst the minor polymorphism H63D, was found in 14% (in a pooled cohort of 170,066). Similarly, Branhagen et al. (2002) reiterate these findings by suggesting that one in 250-300 white persons is homozygous for the haemochromatosis gene mutation and add that one in 10 is a carrier. Douabin et al. (1999) spoke of 10% to 12% of people from a European decent being heterozygous for HH, with 1 in 300 being homozygous. Gochee et
al., (2002) state that the polymorphism in HFE, H63D, has a higher prevalence in the general population (mean allelic frequency ~14%) and is less subject to geographic variation, but it seems to have no clinical penetrance.

Adams et al., (2005) analysed the results of 99,711 individuals in the Hemochromatosis and iron Overload Screening (HEIRS) study in the USA. In a subset of Caucasian participants, 3359 men and 2416 women had prevalence of potential iron-loading HFE genotypes (defined as C282Y homozygote, C282Y/H63D compound heterozygote, or H63D homozygote) was 10% and 12% in men and women, respectively.

3.6 Prevalence in clinically recognised Hereditary Haemochromatosis

Having examined the prevalence of the disease in the general population, the prevalence of the disease in the haemochromatosis population was reviewed. In 1935 Sheldon opined that “it may be accepted that Haemochromatosis is a rare disease.” In 1955 Finch and Finch raised the profile of Haemochromatosis by suggesting that idiopathic Haemochromatosis is present “once in about 7000 hospital deaths” and in general “once in about 20,000.” In the mid 1990’s Feder et al. (1996) stated that HH “affects some 1 in 400 and has an estimated carrier frequency of 1 in 10 individuals of Northern European descent.”

In the 2010 EASL Clinical Practice Guidelines for HFE Hemochromatosis, a meta-analysis of 32 studies with 2,802 individuals with iron overload (clinically recognised haemochromatosis) is put forward demonstrating the prevalence of C282Y homozygosity (C282Y/C282Y) was 80.6% (in a pooled cohort of 2,802) and compound heterozygosity
(C282Y/H63D) was 5.3% (see Appendix 5 for the prevalence of C282Y homozygosity and C282Y/H63D compound heterozygosity in clinically recognized hemochromatosis, J hepatol: 2010).

The prevalence of these genes was analysed by this thesis. Merryweather et al. (2000) note that the frequency of C282Y in Northern Europeans is “between 5 % and 10%” with C282Y homozygotes “accounting for between 1/100 and 1/400.” These figures compare with that proposed by Adams et al. (2005) indicating a prevalence of HH of 1/220 and 1/250.

Geographically, there are disparities across Europe’s regions. Fig 3.8 adapted from European Association for the Study of Liver disease (EASL) shows such disparity in the C282Y allele (mutation).

Fig. 3.11 Frequency of the C282Y allele in different European Regions

Source: EASL Clinical practice guidelines for HFE Haemochromatosis (2010)
3.7 Penetrance of the disease

Penetrance is the percent frequency with which a dominant or homozygous recessive gene or gene combination manifests itself in the phenotype of the carriers (www.merriam-webster.com) or the proportion of individuals of a particular genotype that express its phenotypic effect in a given environment.

The penetrance of the disease was established to determine who may in fact be susceptible to actual iron overload and thereby warrant an echo being performed. This question is at the centre of this thesis problem statement.

From the data assessed in this literature review, the general consensus is quite variable. Edwards et al. (1998) state that “although the time required to become iron loaded is variable, it is clear that most homozygotes will eventually become symptomatic.” It is worth pointing out this case is so only if left untreated, as once diagnosed, symptoms can be arrested if appropriately managed.

According to the Irish Haemochromatosis Association (2013) HH has incomplete penetrance which means that not all those with the HFE gene and its associated mutations have developed the disease but only carry mutations of the HFE gene. As pointed out earlier, they do not have HH until both the phenotype (iron overload) and genotype are identified.

Neghina and Anghel (2011) completed a comprehensive meta-analysis examining 3,572 article titles pooled from 43 study populations. They found that phenotypic penetrance could not be extrapolated to the population in general. Beutler (2001) proposes that in every disorder, individuals with the equivalent genotypes have different clinical phenotypes even
though their mutant genotype is identical and suggests that there are three potential explanations for the variable penetrance, namely: epigenetic, environmental and genetic.

Although the prevalence is high the actual penetrance or risk of developing iron overload is low. According to the Oregon Health Sciences University, evidence suggests that of the C282Y polymorphism group, 60-90% is susceptible to iron overload disease but the risk is not well defined. Also compound heterozygotes (C282Y/H63D) have a very low risk of developing iron overload; patients heterozygous for C282Y (C282Y/n) or H63D (H63D/n) have a very low risk of developing iron overload at approximately 0.5-1.0%. Homozygous H63D (H63D/H63D) patients also have a very low risk of developing iron overload: less than 0.2%.

Brissot et al. (2008) state that

“some confusion in the literature is related to an imprecise definition of hemochromatosis penetrance. It has become clear that the full-blown form of the disease (especially with cirrhosis) is rare (probably only a few per cent of C282Y homozygotes).”

He goes on to state that

“C282Y heterozygosity, H63D heterozygosity and H63D homozygosity may not be responsible for increased plasma iron parameters in the absence of cofactors accounting for disturbed iron metabolism (especially alcoholism or polymetabolic syndrome),” (Brissot et al., 2008).
Fig. 3.12 Phenotypic variability of HFE (type 1) HH. 5 scale grading.

Tf Sat (transferrin saturation) = 45 %: ferritin = 300 ug/L (male): 200 ug/L (female). Quality of life symptoms = asthenia, arthropathy; Life = life threatening symptoms = cirrhosis, diabetes, Cardiomyopathy.

Source: Adapted from: Brissot et al. (2008)

Brisson et al. (2008) provide a phenotypic five scale grading Fig. 3.9. It lends clarity to penetrance in HH. Using the scale, it would imply that “50% of all C282Y homozygotes may present a phenotypic profile (grade 2) justifying venesection therapy.”

3.8 Chapter Summary

In conclusion, there are a large number of HFE polymorphisms, however, according to Adams (2015) only two: C282Y and H63D appear to be clinically significant. HH is a disease caused by a mutation in C282Y, H63D or both which causes iron overload. Its prevalence is high in Ireland. Although studies differ, prevalence is somewhere between 1 per 220 to 250 individuals is commonly acknowledged. Although penetrance is difficult to
quantify, its penetrance is generally thought to be low. The hemochromatosis mutation is common but the hemochromatosis disease is rare (Finch and Sheldon; 1955).

“The physiologic capacity to excrete iron is very limited. Thus, body iron content is regulated almost entirely by controlled absorption. Normal iron homeostasis is maintained by absorption of iron from the diet that precisely balances iron loss,” (Barton et al., 2011).

This can cause organ damage by deposition of iron in the form of ferritin which will be considered in more detail in the following chapter on Blood, Iron and Hepcidin.
Chapter 4: Connecting Hereditary Haemochromatosis to Blood, Iron and Hepcidin

4.1 Introduction

As stated in the previous chapter, Haemochromatosis is an iron overload disorder. As iron is transported by blood, an understanding of the blood and its constituent parts in relation to HH needed to be elucidated. This would lead to a better understanding of the mechanism of iron overload; what indeed were the best biochemical markers to use when assessing iron overload and thereby selection of patients for echo should iron overload be the confirmed diagnosis. Therefore understanding blood, iron biochemical markers and the mechanism for iron overload is important in relation to data selection from the retrospective patient data records audit and the key data reviewed as part of this thesis.

4.2 Blood: its Composition and Functions

Blood is a connective tissue and as its name suggests it connects cells of parts of the body to the environment outside of the body. On average, a 70 kg man would have 5.6 litres of blood and represents about 7% of body weight.

The individual components of blood are red cells, white cells, platelets and plasma. Blood plasma is a straw coloured substance. This liquid blood component is:

“a mixture of water, sugar, fat, protein, and salts. The main job of the plasma is to transport blood cells throughout your body along with nutrients, waste products, antibodies, clotting proteins, chemical messengers such as hormones, and proteins
that help maintain the body's fluid balance,” (American Society of Hematology; http://www.hematology.org/Patients/Basics/).

It represents approximately 55% of total blood volume. The remaining 45% of blood volume is represented by cells i.e. red blood cells (RBC’s) also known as erythrocytes, white blood cells also called leukocytes and platelets also called thromboocytes (see Fig. 4.1).

**Fig. 4.1 Components of the blood: Plasma 55% and cellular elements 45%.

Source: (www.blood.co.uk)**
The main function of white blood cells are to defend the body against infections i.e. ingest pathogens and destroy them; produce antibodies to destroy pathogens and produce antitoxins to neutralise toxins produced by pathogens.

“Unlike red and white blood cells, platelets are not actually cells but rather small fragments of cells. Platelets help the blood clotting process (or coagulation) by gathering at the site of an injury, sticking to the lining of the injured blood vessel, and forming a platform on which blood coagulation can occur,” (American Society of Hematology; www.hematology.org/patients/basics/).

The main function of red blood cells is to transport oxygen from the lungs and deliver it to other tissues and to transport carbon dioxide from other tissues back to the lungs. Red blood cells contain:

“a special protein called hemoglobin, which helps carry oxygen from the lungs to the rest of the body and then returns carbon dioxide from the body to the lungs so it can be exhaled. Blood appears red because of the large number of red blood cells, which get their color from the hemoglobin,” (American Society of Hematology; www.hematology.org/patients/basics/).

The distribution of iron within the blood can be seen in Fig 4.2.

![Iron Distribution](image)

**Fig. 4.2 Iron Distribution in the body in males, females, children.**

**Source:** Adapted from U.S. Centre for Disease Control and Prevention
4.3 Iron and Iron Overload

Comprehending the metabolism of iron underpins any consideration of its toxic effects on the organs. So where it comes from, how it enters the body, what the body does with it and how the body gets rid of it are all relevant questions for this thesis.

Dublin’s Ha’penny Bridge was Ireland’s first iron bridge; this same metal iron comprises of 6.2% of the earth’s crust (Earnshaw and Greenwood, 2010). It is abundant in nature and forms strong bonds with oxygen. “Not long after the Big Bang, iron began to play a central role in the Universe and soon became mired in the tangle of biochemistry that is the prima essentia of life,” (Sheftel et al., 2011). It is found in everyday use; from pots to magnets.

This same metal is also fundamental to human life and without it we would die. Iron is “essential for a wide range of vital cellular functions such as oxygen transport, energy production, and cell division. For this reason, iron homeostasis is strictly regulated at both the systemic and cellular levels in order to keep iron protein-bound,” (Gammella et al., 2015).

Hentze et al., (2010) explain this iron metabolism quite simply as being “balanced by two regulatory systems, one that functions systemically and relies on the hormone hepcidin and the iron exporter ferroportin, and another that predominantly controls cellular iron metabolism through iron-regulatory proteins that bind iron-responsive elements in regulated messenger RNA’s,” (Ribonucleic Acids).

Hentze then goes on to liken the two systems functioning and co-ordinating together like a biological “tango.”
Iron (atomic weight 55.85; atomic number 26) is a *d*-block transition metal (Clugston and Fleming, 2000) that is tolerant of biological redox reactions, having an ability to donate and accept electrons. This means “as a first-row transition element, iron has incompletely filled *d* orbitals and can form a range of oxidation states. The most common oxidation states of iron are Fe II (*d*⃣) and FE III (*d*⃤),” (Outten and Theil; 2009). These common oxidation states allow for their incorporated use in the biological pathways of respiration, photosynthesis and nitrogen fixation.

However, as well as being fundamental to human life, this only holds true at certain concentrations and the converse also holds true: greater than 40 mg/kg of elemental iron is toxic to humans (Manoguerra *et al.*, 2005), which means the human body must have the capability to transform iron within the body to a form that is non-toxic. So, in essence iron ably assists the body with its cellular redox balance.

So where does this potentially life-threatening iron come from? Back to the Universe question; iron is present in the soil which nourishes the plants (photosynthesis) which in turn nourish the animals. Humans eat plants and animals and thereby ingest this iron through the food chain. Bacon (2011) suggests that a western diet includes about 10 to 20 mg of iron from heme compounds of which we absorb 1 to 2 mg see Fig 4.3. However, HH patients can absorb up to 3-6 mg per day and unabated this could build up to overload levels.
Fig. 4.3 High level review of iron and its lifecycle in the human body.


To answer the next two questions in the opening paragraph (what does the body do with the iron and how the body gets rid of it), the full lifecycle of iron in the body was investigated.

Iron’s lifecycle in the human body begins when ferrous iron, also known as heme iron, is absorbed in the intestine through binding with Transferrin. Then transportation to the spleen, liver and bone marrow occurs. Some of the transferrin binds to form haemoglobin in the
bone marrow. The red blood cells (RBC’s) contain 60 to 80 percent of a human body’s iron and 20 to 30 percent of iron is also stored as hemosiderin in the spleen, liver and bone marrow, (Damjanov, 2000) see Fig. 4.4.

Roughly 10-20mg of iron is ingested into the human body through our food chain.

Fig. 4.4 Iron Metabolism.

Source: Adapted From Damjanov, (2000).

Iron is an essential “component of haemoglobin, myoglobin and enzymes necessary for normal cell proliferation,” (Horwitz and Rosenthal, 1999). To elaborate on this Ross et al.,
(2014) state that iron is an essential component of haemoglobin, an erythrocyte protein that transfers oxygen from the lungs to the tissues. Aggett, (2012) describes iron as a component of myoglobin, a protein that provides oxygen to muscles and supports iron metabolism, while Murray-Kolbe, (2010) state that iron is also necessary for growth, development, normal cellular functioning and synthesis of some hormones and connective tissue as it encodes DNA.

Abnormal iron deposition in the cells causes oxidative damage. Serum iron, transferrin saturation, and ferritin have been used widely to assess iron status (Brugnara, 2003). Iron (stored as ferritin and hemosiderin) has no major excretory mechanism in the body apart from blood loss. Brisscot et al. (1981) suggest that there is a rapport between serum ferritin and total body iron stores and these markers co-relate strongly with hepatic iron stores. This iron (1mg of iron per day) is needed to counteract the iron losses through sweat, shed via the skin and through the gastrointestinal tract. McCance and Widdowson, (1937) stated that there is compelling evidence that in human beings iron excretion is greatly restricted (see Fig. 4.5).

Fig. 4.5 Iron overload, pathophysiology. Source: www.cdc.gov
So, in conclusion iron is required to sustain the human body. It is ingested, bound and transported in the body via transferrin. Its storage occurs in ferritin molecules but there is no physiologic mechanism for excretion. Therefore, it is paramount that iron absorption and iron use are balanced through iron homeostasis.

### 4.4 Iron Homeostasis

Iron overload is caused by a genetic defect causing people to absorb excessive amounts of dietary iron. The normal iron balance is maintained by a series of closely regulated by proteins. In normal subjects, absorption of iron from the gut is inversely correlated with iron stores (Baur, 2009). Iron is relatively difficult to absorb from the diet, resulting in only about 10% of dietary iron (approximately 1 mg – 2mg) being taken in as shown in Fig. 4.6.

![Fig. 4.6 Normal iron homeostasis in humans (left) and it’s homeostatic failure in haemochromatosis (right). Source: Pietrangelo, 2005 Hereditary Hemochromatosis, doi:10.1016/j.bbamer.2006.05.013](image-url)
The restriction of iron is balanced by a highly complex iron homeostasis system. Siah et al. (2006) state iron absorption is dependent on the body’s iron stores, hypoxia and rate of erythropoiesis. Particular methods are used both systemically and cellularly to maintain iron homeostasis. Systemic iron is regulated at the transcriptional level and cellular iron balance is regulated by a post transcriptional mechanism (Wang et al., 2011). Iron in the body is bound to the protein transferrin. This non-saturated state ensures that iron remains in a non-toxic form as stated by Outten and Theil, (2009) as follows:

“In the circulation, iron is safely transported by transferrin (Tf), whose binding capacity is normally not fully saturated. Body iron homeostasis is regulated by the interaction of the liver-derived peptide hepcidin and its receptor, the iron exporter ferroportin.”

Hepcidin is discussed later in this chapter with ferroportin will be reviewed below.

Cellular iron uptake occurs as a result of receptors on the cell’s plasma membrane. These receptors bind iron-transferrin complexes and then allow them to enter the endosomes. Once the endosomes take in the iron-transferrin complexes separation occurs. Iron is then moved to the inner part of the cell. The transferrin that remains after the separation from iron is called apotransferrin.

This iron-free apotransferrin is able to start the iron binding process again, binding to and transporting iron atoms into the interior of the cells. “The HFE protein associates with the transferrin receptor and prevents internalisation of the iron-transferrin complex into cells,” (Gross et al., 1998), that is the HFE protein effectively stops iron uptake. However, in the event that there is a mutation in the HFE gene as in HH, “the mutant protein does not
associate with the transferrin receptor and does not dampen the iron uptake by the cells,” (Gross, et al., 1998).

Ferroportin (FPN) is an iron exporting protein and “plays an essential role in iron homeostasis at the systemic level” (Samira et al., 2015). Macrophages (white blood cell, antigen phagocytization and protein secretion that regulate cells involved in immune responses) and entrocytes (hyperpolarized epithelial intestinal absorptive cells) release iron in the plasma, causing iron overload. Remember erythropoietic needs are fulfilled primarily, however, entrocytes continue to transfer unneeded iron from digested matter into the bloodstream, instead of retaining it in the form of ferritin see Fig. 4.7. When this normal balance mechanism is affected iron accumulation occurs.

![Fig. 4.7 Normal iron homeostasis. Uncontrolled release of iron into the plasma. Source: engl j med 350;23 (www.nejm.org June 3, 2004)](image_url)
Practice guidelines from the Bacon *et al.* (2011) on behalf of the American Association for the Study of Liver Disease (AASLD) state that Hereditary Haemochromatosis evolves in a series of three stages as opposed to the five stages put forward by (Jacobs *et al.*, 2007) in chapter two, however, the overall description of the stages are similar:

1. **Clinically insignificant iron accumulation at approximately 0-20 years of age which is associated with 0-5g parenchymal iron storage.**

2. **Iron overload without disease at approximately 20-40 years of age which is associated with 10-20g parenchymal iron storage.**

3. **Iron overload with organ damage at approximately 40 years of which is associated with more than 20g parenchymal iron storage.**

As previously mentioned, duodenal (intestinal) iron uptake increases and iron overload begins to occur in HH patients: but how is this evaluated and diagnosed? Bacon *et al.* (2011) suggests “the initial approach to diagnosis is by indirect markers of iron stores... namely TS or unsaturated iron-binding capacity and serum ferritin.” Transferrin saturation (TS) is calculated using a ratio of serum iron to total iron-binding capacity.

Franchini and Veneri (2005) reiterate this and state that transferrin saturation and serum ferritin are the most reliable tests for detecting individuals with HH. “Serum ferritin has less biological variability than TS, but it has a significant false positive rate because of elevations related to inflammation.” It is worth noting that as Bacon *et al.* (2011) reveal:

“ferritin can be elevated in the absence of increased iron stores in patients with necroinflammatory liver disease (alcoholic liver disease [ALD]), chronic hepatitis B
and C, nonalcoholic fatty liver disease (NAFLD), in lymphomas, and in patients with other non-hepatic chronic inflammatory conditions.”

Cherfane et al. (2013) also note that abnormal iron study results also occur in many liver and hematologic diseases. The normal and abnormal ranges for TS and serum ferritin are outlined in Table 4.1 and 4.2 respectively. Hentze et al. (2010) state:

“in humans, plasma transferrin is normally about 30% saturated with iron. A transferrin saturation <16% indicates iron deficiency, whereas >45% saturation is a sign of iron overload. When saturation exceeds 60%, non-transferrin-bound iron begins to accumulate in the circulation and to damage parenchymal cells.”

<table>
<thead>
<tr>
<th>Transferrin saturation (%)</th>
<th>Interpretation</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;16%</td>
<td>LOW</td>
<td>Consider iron deficiency</td>
</tr>
<tr>
<td>16-45%</td>
<td>NORMAL</td>
<td>Reassure patient that he/she does not have iron overload. Return to usual care</td>
</tr>
<tr>
<td>&gt;45%</td>
<td>ELEVATED</td>
<td>Proceed with serum ferritin tests and additional workup as warranted</td>
</tr>
</tbody>
</table>

Table 4.1 Interpreting the results of a fasting transferrin saturation (TS) test.

<table>
<thead>
<tr>
<th>Serum Ferritin Levels (ng/ml)</th>
<th>Interpretation</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;200 for premenopausal females OR &lt;300 for males</td>
<td>NORMAL</td>
<td>Recheck every 2 years and reassure patient that he/she does not have iron overload.</td>
</tr>
<tr>
<td>200-300 for post-menopausal female</td>
<td>BORDERLINE ELEVATION</td>
<td>Consider other factors in making the recommendation to treat or observe.</td>
</tr>
<tr>
<td>&gt;200 for premenopausal female OR &gt;300 for postmenopausal female OR &gt;300 for male</td>
<td>ELEVATED</td>
<td>In the absence of other causes, removal of iron via phlebotomy is indicated. Confirmation of hemochromatosis is warranted.</td>
</tr>
</tbody>
</table>

Table 4.2 Interpreting serum ferritin test results in patients with elevated fasting Transferrin Saturation.

Jeffrey et al. (1999) state that “transferrin saturation has a sensitivity of greater than 90% for hemochromatosis when a phenotypic case definition is used.” In context, Cardoso et al, (2014) state “the sensitivity of a test is defined as the proportion of people with the inherent disease who test positive (true-positive)”. The demonstration of a high serum iron, transferrin-saturation (serum iron/total iron binding capacity) in excess of 60% in post-menopausal women or 60% in men and 50% in premenopausal women with an elevated serum ferritin is indicative of HH. The combination of elevated transferrin saturation and an elevated serum ferritin is associated with a sensitivity of 0.94 and a specificity of 0.86 in the detection of early hemochromatosis (John Hopkins Medicine https://gi.jhspson.org). In context, Cardoso et al, (2014) state “the specificity of a test is the proportion of people without the disease that have a negative test (true-negative).”

Once elevated serum and/or elevated transferrin saturation has been determined, confirmation of Haemochromatosis is warranted and a genetic test will be performed. If both the phenotype and genotype are present a phlebotomy programme will be commenced. This phlebotomy (also known as bloodletting or venesection) programme typically requires approximately 15 phlebotomies, each removing 450 ml to 500 ml of blood. Each 500 ml of blood extracted removes approximately 200 mg of iron. The goal is to reduce the ferritin level to below 50 ng/ml (Assi and Baz; 2014). The affects of phlebotomy have been researched by The U.K. Haemochromatosis Society. They state as the number of phlebotomies increase, “serum ferritin decreases steadily” and the “transferrin saturation remains high until iron deficiency occurs,” then plummets. Fig. 4.8 illustrates how therapeutic phlebotomy may affect blood iron.
The phlebotomy process is described simply in Fig. 4.9 showing three steps. Red blood cells are removed by phlebotomy which in turn stimulates the bone marrow to produce RBC’s (erythropoiesis) thereby reducing iron stores.
4.5 Serum Ferritin

Ferritin was discovered in 1937 by Laufberger. He noted the presence of ferritin in the spleen and liver of the horse (Granick, 1943). In 1946 Schade and Caroline showed the antimicrobial effect of siderophilin J, the beta-1 iron-binding globulin component of plasma and human serum. This protein siderophilin was later changed to ferritin and ferritin is the best transporter protein for iron. Ferritin is a high-molecular-weight protein that contains approximately 20% iron and present in nearly all of the body’s tissues. It acts as an iron store in hepatocytes and reticuloendothelial cells. However, in the heart “iron is deposited predominantly in myocardial cells, rather than in the interstitium” (Fitchett 1980). It is also present in the serum acting as a surrogate for normal iron stores. Addison et al., (1972) ably
demonstrated that “serum ferritin was elevated in patients with iron overload and decreased in patients with iron deficiency diseases.”

Elevated plasma iron is represented by elevated transferrin saturation whereas elevated tissue iron is represented by elevated serum ferritin. “The plasma iron overload is followed by a second phase, one that involves progressive accumulation of iron in the parenchymal tissues of the liver and other organs, as heralded by rising serum levels of ferritin,” (Pietrangelo, 2004).

Jacobs and Worwood, (1975) proposed an assay of serum ferritin might lead to a “useful and convenient method of assessing the status of iron storage.” Morrison et al. (2003) state that marked elevation of serum ferritin level has been associated with histologic evidence of iron deposition.

Worwood, (1986) states that ferritin is a soluble protein that provides the primary form of iron storage in the body. It has an apoprotein shell encapsulating a core of iron in the form of ferric hydroxyl-phosphate. It has two types of subunits; H and L. H (heavy) subunits are found in the heart in the acidic isoferitins (Arosio: 1978; Powell et al.; 1975; Wagstaff et al., 1982). The “H refers to the original isolation of isoforms of ferritin from human heart, which are rich in the H subunit,” whereas the L (light) subunits “refers to ferritin isolated from human liver, which is rich in a lighter subunit,” (Wang et al., 2010). Iron is stored either as ferritin or as hemosiderin. The former is water-soluble; the latter is water-insoluble (Beutler et al., 2001).

Adams et al. (1999) state that correlation between serum ferritin and total body iron stores with hepatic iron concentration and the amount of iron removed by venesection is clear. This
response to venesection is no longer required as evidence of iron overload due to the existence of genetic testing. Serum iron can be raised as a result of chronic inflammation and histiocytic neoplasms. Wish (2006) mentions that “Serum ferritin is an acute-phase reactant,” meaning that inflammatory states may increase serum ferritin levels suggesting iron overload. This is an important point to note in HH patients because the serum ferritin may be raised as a result of an inflammation process at work and not necessarily iron overload as discussed earlier in this chapter.

4.6 Hepcidin

Hepcidin is the body’s police mechanism for iron absorption. As can be evidenced in the following, hepcidin is easily detected and lack of hepcidin is a leading cause of iron overload.

Pantopoulos et al. (2012) describe hepcidin as “the master regulator of both dietary iron absorption and systemic iron trafficking and homeostasis.” Hepcidin (also known as liver-expressed antimicrobial peptide (LEAP-1)) is a protein which “can be detected in both human blood and urine,” (Krause et al., 2000). According to Wish (2007) “if storage iron is elevated then the liver synthesises hepcidin” to assist in lowering iron levels.

“Hepcidin is an antimicrobial peptide produced by hepatocytes in response to inflammatory stimuli and iron,” (Krause et al., 2000; Park et al., 2001; Pigeon et al., 2001). This regulatory hormone polices iron transport into the plasma by binding to ferroportin on the “enterocytes, macrophages, hepatocytes and other cells,” (Nemeth et al., 2004).
Iron overload occurs when the presence of hepcidin is deficient in the blood as noted by Sebastinai and Pantopoulos (2011): “hepcidin deficiency... is associated with uncontrolled dietary iron absorption and progressive tissue iron overload.” This was reiterated by Al Wayli et al., (2011) who state: “Hepcidin is an iron regulatory hormone that inhibits ferroportin-mediated iron export from enterocytes and macrophages.”

Inappropriate levels of hepcidin, the iron hormone, appear now as the central pathogenic event in all forms of haemochromatosis. The link made by Pigeon et al., (2001) between hepcidin and iron metabolism changed how HH was looked at. Dallalio et al., (2003) concur stating “the serum hepcidin concentration exhibited a statistically significant correlation with serum ferritin concentrations.”

The 25-amino acid antimicrobial peptide hepcidin has been shown to play a major role in iron homeostasis (Park et al., 2001). In a mice study (Nicholas et al., 2001), the mice lacking this peptide showed progressive iron accumulation whereas over-expression of hepcidin in mice leads to decreased iron stores and severe microcytic hypochromic anemia at birth. The upstream stimulatory factor 2 (USF2) knockout mouse, which does not express hepcidin was found to have iron overload resembling HH where iron deposition was significant in the liver, pancreas and heart.

4.7 Chapter Summary

In conclusion, Hereditary Haemochromatosis is an iron overload disorder caused by a mutation in the HFE gene. Iron metabolism involves maintaining a fine balance. It has been
established that iron overload can be determined by biochemical surrogates for iron; serum ferritin and transferrin saturation. Hepcidin is fundamental in maintaining this balance.

“The deficient production of hepcidin causes a situation resembling a tap that cannot be turned off. Intestinal cells and macrophages continue to release unneeded iron, depleting the stores in these cellular compartments and overloading the circulatory pool.” (Pietrangelo, 2006).

Phlebotomy is the approved treatment for iron overload. If phlebotomy treatment is not undertaken then according to Cairo et al. (1997) the reserve iron in HH is “primarily deposited in parenchymal cells, with reticuloendothelial cell accumulation occurring very late in the disease,” as opposed to deposited in the reticuloendothelial cell (RE iron) and then parenchymal cells in secondary iron overload like that caused by transfusional iron overload. What this actually means is that a:

“Distinction among these causes of iron deposition is clinically important because parenchymal iron overload from hemochromatosis may produce significant tissue damage, while the RE iron of transfusions and rhabdomyolysis is of little clinical consequence,” (Siegelman et al., 1991).

While this could imply that the parenchymal cells of the heart could be infiltrated with storage iron leading to more drastic consequences, the actual fact is that the principal features of HH are decreasing over the years as evidenced in Table 4.3. This could be due to earlier diagnosis, treatment with phlebotomy or a number of other factors.
### Table 4.3 Principal Clinical features in Hereditary Hemochromatosis.

**Source:** Bruce *et al.* (2011). Diagnosis and Management of Hemochromatosis: 2011 Practice Guideline by the American Association for the Study of Liver Diseases, Hepatology July: 54(1): pg 328-343

Chapter 5 will now address the link between HH, iron, the heart and what echocardiography can add to the clinical diagnosis and management of HH.
Chapter 5: The Link between Hereditary Haemochromatosis, the Human Heart and Echocardiography

5.1 Introduction

As discussed in chapter three, iron is crucial to human life, specifically to our cellular function. Iron deficiency can limit cardiac function in heart failure patients, a fact demonstrated in two major randomised clinical trials: the FAIR-HF trial and the FERRIC-HF trial (Anker et al., 2009). However, the corollary to this statement is also true: too much iron can also have a negative impact on cardiac function. Gammella et al. (2015) state that “iron accumulation in the heart plays a major role in the process leading to heart failure.” Also Shizukuda et al. (2006) similarly state that “patients with hereditary hemochromatosis (HH) have been reported to develop diastolic functional abnormalities detectable by echocardiography” as a result of the iron overload.

The purpose of this study was to ascertain if an echo was warranted in the HH venesection population of LCH. To form a conclusion on this, a definitive link between iron overload, its effects on the heart in HH patients and the use of echocardiography in diagnosing iron overload as evidenced by the available literature on the subject had to be found.

Firstly, as pointed out in previous chapters, the HH genetic condition causes excessive amounts of dietary iron to be absorbed from the intestine and stored by the organs. The patients attending the LCH venesection clinic showed signs of this as will be evidenced in chapter seven. Over the course of a number of decades, the excessive storage grows to toxic levels as evidenced by Barton et al. (2011): “When storage mechanisms are overwhelmed,
Iron in low-molecular weight forms can catalyze free radical reactions.” The oxyradicals can damage nucleic acids, cellular lipids, proteins and carbohydrates. This point was reiterated by Young et al. (1994) stating that “free iron is a potent promoter of hydroxyl radical formation that can cause increased lipid peroxidation and depletion of chain–breaking antioxidants.” Chan et al. (2015) suggest that iron-overload generates reactive oxygen species (ROS) production which in turn encourages apoptosis in cardiomyocytes via mitochondrial pathways. Fig. 5.1 summarises this degeneration and clearly lays out that damaged cardiomyocytes fibrose and lead to heart failure.

![diagram]

**Fig. 5.1** Roles of iron in the pathologic progression leading to cardiac dysfunction. Excess iron, from systemic overload (e.g. thalassemia) or mislocalization (e.g. FRDA) can catalyse ROS.
As stated in the previous chapters, Haemochromatosis is an iron overload disorder. In the context of the heart, this disorder is hard to comprehend as it is primarily a gastroenterology problem. A review of literature demonstrates a number of authors believe the liver is most affected by HH because of its large blood flow. Obviously the heart’s function is blood flow, however, some writing on the matter conclude the issue of iron overload in the heart is due to a combination of the presence of more mitochondria and the heart’s inability to clear toxins like the liver. Doroshow et al. (1980) for example state: “given the heart’s high need of energy, cardiomyocytes are rich in mitochondria and consume large amounts of oxygen; cardiomyocytes have low levels of antioxidant enzymes.” Thus, the heart becomes a victim of iron, resulting in iron overload. This iron overloading had not manifested in the LCH cohort examined as evidenced in chapter seven.

The literature research regarding the reasons and effects of iron overload and the heart are central to understanding the iron overload cardiac relationship. In this chapter a number of opinions, some similar and some contradictory, are investigated. A sample from these opinions is highlighted below to give a sense of latitude and scope of judgment on the subject. Understanding and awareness of these opinions is central to this thesis and the objective is to provide comprehension and clarity on the subject.

In this chapter, the general introduction sets the scene, followed by iron’s effects on the heart, a physiological outline of the heart, phlebotomy and the heart, echocardiography and HH, and finally a short conclusion is provided.
5.2 Latitude and Scope of Judgment on iron overload and the effects on the Heart

Witness these statements taken from the literature review as a sample of the similar and contradictory conclusions regarding this subject:

“Little is known about the relationship of heart iron content to mortality. This is partly because endomyocardial biopsy is impractical in routine clinical practice and is not a useful indicator of heart iron as a whole owing to very uneven iron distribution in the heart,” (Barosi et al., 1989).

Modell and Berdoukas (1984) showed that post-mortem measurements of iron concentration in many organs, even in patients dying of heart failure, heart iron was only a fraction of that in the liver.

Cutler et al. (1980) stress the importance of early recognition of Haemochromatosis heart disease and state that “hemochromatosis represents the only cause of a restrictive Cardiomyopathy that is potentially reversible by medical therapy.”

Olson et al. (1989) assert that “Cardiac hemochromatosis represents a storage rather than an infiltrative disease. This fact was proven histologically because iron was present within the sarcoplasm.” Olson et al. also stated the extent of the iron varied inversely with ventricular function.

“Complications, which are preventable by iron depletion therapies, can be fatal and include liver cirrhosis, cancer, diabetes, hypogonadism, heart failure and arthritis,” (Hentze et al., 2010).
Iron Overload Cardiomyopathy (IOC) is the term used to describe the cardiac dysfunction that results from the accumulation of iron in the heart whether from primary or secondary hemochromatosis (Kreminastonis et al., 2011). Also as stated by Cheng and Lian (2013):

“Iron overload cardiomyopathy (IOC), defined as the presence of systolic or diastolic cardiac dysfunction secondary to increased deposition of iron, is emerging as an important cause of heart failure due to the increased incidence of this disorder seen in thalassemic patients and in patients of primary hemochromatosis.”

“Several diagnostic methods have been developed in order to detect myocardial iron overload as early as possible, thereby averting a process that can lead to Cardiomyopathy, heart failure, and untimely death,” (Gammella et al., 2015).

T2-star magnetic resonance (MR-T2*) for example is a diagnostic tool for evaluation of cardiac iron status and detection of early global ventricular dysfunction (Lekawanvijit and Chattipakorn, 2009).

Finally, Niederau et al. (1996) evaluated a cohort of 251 patients over a number of years and concluded that early diagnosis of HH, along with therapy prevent the unfavourable consequences of iron overload. The LCH population cohort of 833 patients seems to concur with this conclusion as they too showed no signs of iron overload.

These sample opinions are central to this thesis problem statement of whether or not HH should be a valid indication for an echo for patients in a HH venesection population and what can clinicians conclude from an echo if it was warranted.
5.3 Cardiac Anatomy and Physiology

In order to understand the heart’s function and how it is affected by iron overload, the cardiac anatomy and physiology will briefly be described.

Schurig et al. (1997) describes the basic cardiac anatomy and haemodynamics. Fig. 5.2 shows the heart has four chambers: two atria and two ventricles. The right and left atria are responsible for receiving blood from the body and are low pressure chambers. The ventricles are the chambers responsible for pumping blood out of the heart to the body.

![Fig. 5.2 The Normal Heart Structure](source: www.DrHeart.in (2010))
The right atrium takes delivery of un-oxygenated blood from the body via the inferior vena cava (IVC), superior vena cava (SVC) and from the heart muscle via the coronary sinus with mean pressures ranging from 0-6 mmHg. The left atrium takes delivery of oxygenated blood from the lungs via the four pulmonary veins (the venous system). The maximum mean pressure at this juncture is 12 mmHg.

The ventricles pump blood into body. The right ventricle takes delivery of un-oxygenated blood from the right atrium and pumps it into the low pressure system of the lungs via the pulmonary artery, where according to Jones and Blackwood (1992) “the systolic pressure should not exceed 30 mmHg.”

The left ventricle takes delivery of oxygenated blood from the left atrium and pumps blood into the coronary arteries that supply the myocardium (during ventricular relaxation) and into the high pressure system (systemic circulation) via the aorta. This cardiac cycle takes 0.8 seconds. The pumping out of blood is called systole and hence systolic function and the relaxation of the ventricle is called diastole or diastolic function.

Ventricular systole comprises of two phases: isovolumic contraction (phase 2) and rapid ventricular ejection (phase 3). Ventricular diastole equally has two phases: isovolumic relaxation (phase 4) and rapid ventricular filling (phase 1). This is illustrated in Fig 5.3.
Fig. 5.3 Cardiac Cycle mechanics demonstrating the atrial and ventricle systolic and diastolic functions.

**Source:** Silverthorn *et al.* (2009), Human Physiology: An Integrated Approach, Pearson Education.

**5.4 Histology – The walls of the heart**

Having reviewed the haemodynamics of the heart, it is important to review the internal structure and the microscopic anatomy because it is here where the iron deposits. The heart walls comprise of the epicardium, myocardium and endocardium as shown in Fig. 5.4.
Fig. 5.4 Histology – the walls of the heart: epicardium, myocardium, endocardium and pericardial sac.

Source: www.histologyolm.stevegallik.org

The epicardium is the outer layer of the heart. This thin, serous membrane is part of the serous pericardium. The pericardium acts as a protective sac surrounding the heart. The myocardium is the heart muscle layer in the middle. The innermost layer which provides a smooth lining with endothelial tissue is the endocardium. The pericardial sac is a fibrous tissue layer consisting of a visceral or epicardial layer of heart muscle and the parietal pericardial layer lining the pericardial sac.

The relevance of this histology from a HH viewpoint is that HH can lead to a dilated Cardiomyopathy characterized by the development of heart failure and conduction
disturbances due to excess deposition of iron within the myocardium. As referenced by Olson et al. (1987) and Palka et al. (2004) “iron-catalyzed myocardial injury leads to diastolic dysfunction affecting predominantly LV subepicardial layers.” Olson et al. (1987) also describes how this injury begins within the epicardium, then extends to the myocardium and finally reaches the endocardium. Nordberg et al. (2007) state that “Stainable iron assumes a sarcoplasmic localization, but precise subcellular distribution of iron has not been well defined.”

5.5 Histology - The Cardiac Muscle Cell

Cardiac muscle cells are known cardiac myocytes, which are cylindrically shaped cells with sizes ranging between 80-150 μm in length, and 10-20 μm in diameter (Walker and Spinale, 1999). The cardiac muscle comprises of sarcomeres and intercalated discs. Sarcomeres contain actin and myosin filaments essential for the contractile function of the cardiac muscle fibre. The intercalated discs are myocardial muscle cells responsible for conducting the rapidly generated cardiac electrical impulses from cell to cell (see Fig. 5.5).

Fig. 5.5 depicts the anatomy of a cardiac muscle.

(a) Histologic section of Myocardial cells

(b) A cross-section of the internal structure of cardiac muscle cell
There is general consensus in the field regarding the method and location of iron deposition in the myocytes as evidenced by Horwitz et al. (1999) stating:

"as the storage capacity of cardiac cells is exceeded, excess iron becomes released intracellularly as hemosiderin and free iron. This results in the formation of reactive oxygen species, which in turn initiates the processes of lipid peroxidation, membrane permeability alteration, and myocyte death."

**Fig. 5.5 Microscopic Anatomy of Cardiac Muscle.**

**Source:** Olson, L., Edwards W., McCall J., Ilstrup D., Gersh B. (1987)
This process involves a series of different stages: apoptosis, fibrosis and cardiac diastolic dysfunction and dilatation. This creates an iron storage disorder with the potential to damage cardiac tissue and cause functional impairment (Galiuto et al., 2013).

Janower et al. (2007) are also in agreement and state “cardiac involvement in hemochromatosis affects mainly the myocardium: iron overload of the myocytes reduces left ventricular distensibility,” and causes cardiac dysfunction. While Swanton (2003) writes that “increased iron absorption with iron overload results in iron deposition in the myocyte sarcoplasm, with subsequent cell death and fibrosis.” This in turn leads to diastolic dysfunction and later to LV remodeling in the form of a dilated LV.

A similar conclusion was written by Olson et al. (1987) when they assessed autopsies on the hearts of 14 men with Haemochromatosis: “The atria and 10 sites in the ventricles were histologically graded for stainable iron. Stainable iron was exclusively sarcoplasmic.”

Case studies from the Massachusetts General Hospital Weekly Clinicopathological Exercises (1994) revealed that:

“early in the disease process, excess iron is preferentially deposited in the ventricles. This manifests as progressive diastolic dysfunction consistent with restrictive physiology. As the disease progresses and maladaptive remodeling occurs, the LV dilates and systolic dysfunction develops.”

Fig. 5.6 shows an example of both the histological myocardial biopsy and a cross section of a pathology specimen from an iron overloaded heart as described in the previous paragraphs.
Fig. 5.6 Hemochromatosis Histological Myocardial biopsy specimen and Cross section of Dilated Left Ventricle and Right Ventricle.

**Source:** Olson *et al.*: 1994.

**A:** Histologic section of myocardial biopsy specimen showing iron (black stain) within the myocardial cells (arrows). **B:** Gross pathology specimen with the reddish-brown rust appearance and dilated cardiac chambers characteristic of cardiac Haemochromatosis. LV: left ventricle; RV: right ventricle

### 5.6 The Role of Phlebotomy and the heart

The consequences of iron overload described above can be ameliorated by use of phlebotomy which “mobilizes the excess iron stored in cells, decreasing myocardial iron content and improving LV function,” (Dabestani *et al.*, 1984). This was similarly demonstrated in a study which showed reductions in “left ventricular dimensions and improvement in fractional shortening and ejection fraction in 11 primary haemochromatosis patients who underwent
phlebotomy,” (Candell-Riera et al., 1983). Fig. 5.7 shows an actual example of an Echocardiogram of a 55-year-old woman who presented with dyspnea, fatigue and a dilated left ventricle (LV). Her ejection fraction was initially severely reduced and after repeated phlebotomy her heart recovered.

Fig. 5.7 shows an actual example of an Echocardiogram of a 55-year-old woman who presented with dyspnea, fatigue and a dilated left ventricle (LV). Her ejection fraction was initially severely reduced and after repeated phlebotomy her heart recovered.

The figures above show echoes from a 55-year-old woman who presented with dyspnea and fatigue. The figure on the left shows the left ventricle is dilated, with marked systolic dysfunction (ejection fraction = 16%). The figure on the right is an echo six years after repeated therapeutic phlebotomy (LV cavity decreased in size, and systolic function improved: ejection fraction = 69%), Seward et al. (2010).

Alexander and Kowdley (2009) also discuss this fact demonstrating that cardiac involvement occurs late in the course of HFE HH and that venesection or iron depletion can improve
cardiac function before the development of cardiac dysfunction. In fact, they also state that cardiac dysfunction is not prevalent at all due to early diagnosis and treatment of HH. This is an important element in relation to this thesis argument.

5.7 Iron overload and Cardiac manifestations

Beutler et al. (2003) have said that only 1% of homozygotes develop clinical manifestations associated with HH. He also says Clinicians do not encounter many cases of full-blown hemochromatosis. HH occurs late in life as has been previously established: iron overload occurs in the fourth decade of life.

Baur (2009) addresses the clinical manifestations of iron overload and states that they include liver disease in 75% of cases, weakness and lethargy in 74%, skin hyperpigmentation in 70%, diabetes mellitus in 48%, arthralgia in 44%, impotence in 45%, electrocardiographic abnormalities in 31% and heart failure and conduction disturbances in 15%.


Gulati et al. (2014) remind us that iron overload is diagnosed based on serum ferritin >300 ng/ml and a mutation in the HFE gene as previously discussed in Chapter 3. He also goes on to say that diastolic dysfunction, arrhythmias and dilated cardiomyopathy otherwise known as primary iron overload Cardiomyopathy or Cardiac Haemochromatosis are potentially
reversible. These reversible echocardiographic findings associated with advanced stages of Haemochromatosis are also clearly defined by Oh et al. (2006) as a dilated LV with decreased systolic function, normal wall thickness and restrictive diastolic filling.

5.8 Echocardiography

Echocardiography as its name suggests, describes the use of “ultrasound in cardiology in which returning echoes are reflected from the boundaries of cardiac structures,” (Anderson, 2004). Echocardiography began in the 1950s with quite basic equipment. Today’s clinicians have progressed and made enhancements to original techniques for measurement and analysis of the heart using two dimensional, three dimensional, M-mode, spectral, colour and tissue Doppler.

Echocardiography images are quantitatively and qualitatively assessed and the results used by many disciplines. According to the American Institute of Ultrasound in Medicine (AIUM), the use of echocardiography “literally became a life-changing event,” (Meyer, 2004). Echocardiography is safe and provides non-invasive, investigative and diagnostic cardiac data. On average at least 1,200 outpatient echocardiograms are performed in LCH each year.

Poulsen (2001) reminds us that two-dimensional and doppler echocardiography has become a well accented, practical and safe non-invasive method for diagnosis of LV diastolic dysfunction and she also mentions that doppler flow profiles are influenced by several factors including age, heart rate, load conditions and valve heart diseases which must be taken into consideration during evaluation.

The British Society of Echocardiography (BSE) and the American Heart Association (AHA) provide echocardiographers with guidelines and minimum data set requirements required
when evaluating the heart. The BSE recommends this and states that the “minimum data set provides a template against which studies in any department should be audited.” See Appendix 6 for further details.

Palka et al. (2002) debate the usefulness of conventional echocardiography in HH patients with diastolic dysfunction stating that “findings are often not specific.” In contrast to this Stollberger and Finsterer (2001) state storage disorders can lead to cardiac involvement “which can be visualized by echocardiography as all cardiac chambers may be dilated in advanced disease.” They continue to say that echocardiographic findings may also be unspecific and may be overlooked. While Cecchetti et al. (1991) found that “echocardiographic abnormalities were significantly more frequent and marked in subjects with higher iron overload than in those in whom it was lower.”

When an indication for echo on a HH patient is requested, evidence of restrictive type diastolic dysfunction and Cardiomyopathy are sought. Kremastinos et al. (1993) state that the early manifestation of cardiac involvement is the restrictive type diastolic dysfunction detected by doppler echocardiography. Galiuto et al. (2012) suggest that particular attention should be paid to the following when assessing patients with haemochromatosis and other storage diseases:

- Myocardial Texture
- Left Ventricular wall thickness
- Global systolic function (Teichholz and Simpson)
- Regional left ventricular wall motion abnormalities (RWMA)
- Diastolic function
- Valvular function
- Right Ventricular function
These measurements will be described in more detail below in relation to HH.

5.8.1 Myocardial Texture

This refers to the appearance of the myocardium or the myocardial reflectivity/brightness on echocardiogram see Fig. 5.8 A and B.

Fig. 5.8 Echoes of the heart A and B contrasting echogenicity/brightness

Source: www.revespcardiol.org
The heart in Fig. 5.8A appears brighter or more echogenic than the heart in Fig. 5.8B.

5.8.2 Left ventricle wall thickness

“Hemochromatosis appears as a dilated cardiomyopathy with normal wall thickness.” (Klein et al., 1998). In a normal heart, the wall measurement will be symmetrical and measure up to 1.1 cm (11 mm). Echocardiographers use the parasternal long axis view (PLAX) of the left ventricle which allows for M-mode or 2D measurement of the left ventricle walls i.e. the interventricular septum and the posterior (inferolateral) wall. Fig. 5.9 represents the echocardiographic structures that are visualised parasternally and transected. This is an example of one of the methods of measuring the wall thickness and an actual image of wall thickness on 2D m-mode.

The image on the left in Fig. 5.9, depicts the echocardiographic structures visualised along a single line (or M-mode) transmitted by the transducer located on the chest wall and penetrating perpendicularly the structures below. The image on the right demonstrates the method of measurement of the left ventricle cardiac dimensions as recommended by the American Society of Echocardiography.
Fig. 5.9 The parasternal long axis view of the left ventricle (note EKG is an ECG).

Fig. 5.10 Actual normal left ventricular M-mode wall measurements (green lines)

Source: www.stanford.edu

Fig. 5.10 shows an echocardiographic screenshot from the 2D M-mode. The two vertical green lines indicate the measurements being observed. The standard measurements are shown in the top right of the screen and are explained as follows: IVSd – interventricular septum in diastole; IVSs - interventricular septum in systole; LVIDd – left ventricular internal diameter in diastole; LVIDs – left ventricular internal diameter in systole; LVPWd - left ventricular posterior wall in diastole; LVPWs - left ventricular posterior wall in systole.
5.8.3 Systolic function

Left ventricular systolic function is defined by Galiuto et al. (2013) as “the ejection fraction (EF), defined by the fraction of volume ejected during each ventricle contraction.” Schiller and Foster (1996) add to that by stating that “Ejection fraction is probably the most commonly discussed clinical index of left ventricular function.” LV systolic dysfunction arises when the ejection fraction is reduced.

Horwitz and Rosenthal (1999) describe the hemodynamic and morphological abnormalities of cardiac haemochromatosis as either “dilated or restrictive” and state that early dysfunction is characterized by diastolic and restrictive physiology and later disease is manifest by dilated cardiomyopathy with left ventricular systolic dysfunction.” They also add a salient point that “Moderate to severe left ventricular dysfunction is associated with heavy iron deposition in the heart.”

Fig 5.11 is echocardiography’s most frequently used method for calculating systolic function by using LV volume. It is known as the biplane method of discs (modified Simpson’s rule). This technique for estimating EF is recommended by the American Society of Echocardiography and the European Association of Echocardiography.
Fig. 5.11 Simpsons 2D method for calculating Left Ventricular volumes, 4 chamber and 2 Chamber.


Fig. 5.11 shows tracing of the endocardium in systole and diastole. A4C = apical four chamber; A2C = apical 2 chamber; EDV = end diastolic volume; ESV = end systolic volume.
5.8.4 Diastolic Dysfunction

In the context of HH, diastolic dysfunction is an early indicator of a disease process which is underway. Gaasch and Little (2007) describe diastolic dysfunction as

“functional abnormalities that exist during LV relaxation and filling. When such abnormalities cause or contribute to the clinical syndrome of heart failure with a normal LV ejection fraction, it is appropriate to describe the condition as diastolic heart failure.”

Quantitative measurement of diastolic function can be performed by using pulsed wave doppler (PW-doppler) and pulsed wave tissue doppler integral (TDI).

Anderson (2004) suggests that the diagnosis of diastolic heart failure “requires three conditions: (1) presence of signs or symptoms of heart failure; (2) presence of normal or slightly reduced LV ejection fraction (EF >50%) and (3) presence of increased diastolic filling pressure.”

Firstly, the left atrial size is an important indicator of elevated filling pressures and should be measured as part of the diastolic assessment see Fig. 5.12. The left atrial (LA) volume should also be measured at end systole. The LA is largest at this part of the cardiac cycle and gives the accurate measurement of LA volume. Secondly, normal LV diastolic dysfunction can be characterised by left ventricular (LV) mitral inflow. To measure this mitral inflow, a pulsed waved (PW) doppler sample volume is placed at the mitral valve (MV) leaflet tips at end expiration. This inflow is a resultant velocity caused by LV suction and the pressure gradient between the left atrium and left ventricle.
Fig. 5.12 LA Volume Measurement.

Source: http://dx.doi.org/10.1093/ej echoc ard/jeq164 140-147 First published online: 26 November 2010

Fig. 5.12 depicts representative images of the measurement of the left atrial (LA) volume. Picture (A) is an apical four-chamber view; Picture (B) is an apical two-chamber view. Typical measurements are of the left atrial areas in the apical four- and two-chamber views (A1 and A2 respectively), and the left atrial common long axis (L1). LA volume is calculated using area-length method and modified Simpson's method.
5.8.5 Diastolic Filling Pressure

There are two diastolic flow velocity envelopes that can be observed in normal sinus rhythm. The E wave represents early passive filling of the LV, whereas the A wave represents late diastolic active filling of the LV caused by the contraction of the atria (see Fig. 5.13). Impaired relaxation is shown in Fig. 5.14. Abnormal mitral inflow velocities are indicated by the large A wave and the reduced E wave. This indicates reversed transmitral Doppler.

Fig. 5.13 Normal mitral inflow velocities from the left atrium with the pulsed wave (PW) sample volume placed at the leaflet tips in the left ventricle during diastole.

Source: www.echobasics.de
Fig. 5.14 Abnormal mitral inflow velocities are indicated by the large A wave and the reduced E wave.

Source: www.echobasics.de

Mitral annular velocities are another method of measuring diastolic function using Tissue Doppler Integral (TDI); the PW sample volume is placed at the medial and/or the lateral annulus of the mitral valve see Fig. 5.15. The sample volume is placed at or within one centimeter of the annulus. This method is invaluable because it is not dependant on preload.
Fig. 5.15 Normal pulsed wave TDI. The sample volume is placed at the lateral MV annulus

Source: www.echobasics.de

Fig. 5.16 TDI is suggestive of impaired diastolic dysfunction

Source: www.echobasics.de
Fig. 5.16 suggests an impaired diastolic dysfunction as the E’ wave and A’ wave are reversed compared to Fig. 5.15.

In Fig. 5.16, the expected restrictive diastolic dysfunction pattern associated with end stage iron overload is shown. That is: Normal diastolic filling (E > A); delayed relaxation (E < A); and restrictive (E >> A) filling patterns. For more information see Appendix 7.

When assessing diastolic dysfunction (DD), multiple echocardiographic measurements looking at the various facets related to DD i.e. LA size, mitral inflow, TDI etc. should be made so that an informed decision can be reached on the DD. An assessment based on any single parameter related to DD should not be made. Refer to Appendix 8 for a practical approach to the assessment and grading of Diastolic Dysfunction.

5.8.6 The Right Ventricle (RV)

Assessment of the RV is made to establish RV systolic function and size. Right-sided chamber dilatation with left ventricular dilatation can cause biventricular failure which may lead to classic symptoms of heart failure (Bejar et al., 2015).

5.8.7 Valvular Assessment

The valves of the heart (the aortic, pulmonic, mitral and tricuspid) should always be assessed as part of a standard echocardiogram.
5.8.8 Regional Wall motion abnormalities (RWMA)

Galiuto et al. (2013) describes myocardial walls according to the distribution of coronary arteries. The walls are divided into regions or segments and are assessed in terms of their systolic motion and thickening. The regions are scored as having: normal motion, hypokinetic, akinetic, dyskinetic or aneurysmal. RWMA would give an indication as to the severity of iron overload and could also give an indication of LV dysfunction reversal (return of normal LV systolic function) as a result of iron reducing therapy.

5.9 Chapter Summary

The progress in the field of understanding iron overload and the heart has shown substantial leaps in the 150 years since the discovery of Haemochromatosis. The Nicholas et al. (2001) mice iron knock out trial has shown that iron can produce cardiac damage. The literature review has shown that iron overload can be and is very treatable, essentially leaving an individual with HH with a normal life expectancy and little risk of Cardiomyopathy.

Erhardt et al. (1999) stated “as a normal life expectancy of patients with HH can be achieved if iron reduction is initiated early, genetic testing may thus be of great benefit for patients with HH.” Echo is a very useful method of evaluating the heart’s diastolic function and determining if either restrictive or dilated Cardiomyopathy is present.

However, an echo should only be indicated in the event that there is a real risk of iron overload. Shizukuda et al. (2011) found that in subjects with cardiac asymptomatic HH post
conventional phlebotomy treatment, LV diastolic function statistically did not significantly
deteriorate during a 5-year period regardless of their treatment history. If HH caused high
mortality then there would be an expectation that there would be an underrepresentation of
elderly patients with the disease. “The life expectancy of these patients, as measured over
the follow-up period (14 years), was indistinguishable from that of the general population,
corrected for age and sex,” (Niederau et al., 1996).
Chapter 6: Analysis of Hereditary Haemochromatosis

Patient Cohort – Methodology Study

6.1 Introduction

The methodology employed during this study is depicted over the following sections.

6.2 Research Process Design Overview

The research process design overview is shown below in Fig. 6.1 and has nine formal steps.

Fig. 6.1 The Research Process Design Overview for this study and thesis

Steps one to seven of the process are described in this chapter. The final two steps have separate chapters each as they are the focus of this thesis. Data Collection and Review is presented in Chapter 7, the Discussion in Chapter 8, with Conclusions and Recommendations presented in Chapter 9.
6.1.1 Step 1: Framing a Problem Statement

The objective of this first step is to frame a problem statement. This step began with an anomaly being noted: generally, the overall increase in demand for echoes and specifically the exponential increase in demand from the LCH venesection clinic as explained in Chapter 1. Given the fixed capacity for echoes within the department, these increases were resulting in increased waiting lists as capacity requirements had surpassed its finite limit. Allying this issue with patient scheduling on a first come, first served policy, thereby taking no clinical urgency into account, it was believed that there could be serious ramifications for patient welfare and waiting list management.

This judgment in fact was confirmed by initiating an informal, high level investigation of the echocardiography diary for the previous twelve months. This was the preliminary part of structuring the problem statement. With this general information a problem statement was framed and thus formed a potential benefit analysis from which it could determine whether further investigation was worthy.

6.1.2 The Problem Statement

LCH has one echocardiographer with a finite capacity to provide a range of services to the patients and clinicians serving the LCH, Dundalk and Our Lady of Lourdes, Drogheda. The total demand trend on the service had noticeably increased over the past ten years without any service capacity increase in line with Health Service Executive budgetary forces.

This demand trend was noted an initial high level initial investigation of the previous years’ echocardiographic appointments. It detailed that over the past decade there was an
exponential increase in the number of echoes being requested for HH patients from the LCH Venesection Clinic. The demonstrated data trend of increased requests is shown in Chapter 1 Fig. 1.1. Taking 2009 as a baseline (a quantity of 13), 2014 showed a 1354% increase to a quantity of 189.

When the level of service demand was related to the known capacity of the echocardiography department, this increase created a direct amplification in patient waiting lists. Most importantly, as requests were dealt with on a first come first served basis, the relative importance of patient treatment needs was not being taken into account on the backlog waiting list. This could potentially lead to delay of urgent echoes which could be time and clinically critical to a positive patient treatment outcome.

6.1.3 Step 2: Formulation of Potential Study Objective Aims

The objective aim of this study was to retrospectively investigate if an echo was warranted in the HH population at LCH Venesection Clinic. Then, following from the conclusions of this study, recommend to the Gastroenterology department whether the guiding principles of LCH should be retained or revisited if contrary to the conclusions.

The outcome of this study would possibly directly affect the future number of echocardiography requests from the HH venesection clinic and the department’s ability to execute and report on all warranted requests in a clinically safe timeline whilst proactively managing the waiting list.

6.1.4 Step 3: Building an Hypothesis

In order to create an hypothesis for the study to test and have a meaningful and unbiased review of the objective, an assessment of the primary medical field guidelines from the appropriate governing bodies of the British Society of Echocardiography (BSE), the
European Association for the Study of the Liver (EASL) and The American College of Cardiology (ACC) was completed as previously discussed in chapter two. These guidelines from the BSE, EASL and ACC were used as a basis for the thesis’ relevance to current practice and to rationalise and guide the study hypothesis.

In conclusion, the governing body of Echocardiographers, the BSE, have no stated requirement warranting an echo to be performed for HH patients, while the EASL put forward an algorithm stating the need for C282Y homozygous patients only to receive an echo in the event that the patient is symptomatic and has increased Serum ALT, AST and HbA1c. The ACC similarly have an algorithm to ascertain if an echo is warranted but only in patients showing symptoms of IOC, that is “systolic or diastolic cardiac dysfunction secondary to increased deposition of iron in the heart independent of other concomitant processes” (Liu and Olivieri :1994).

Using this evidence as a starting point the following hypothesis was put forward for testing:

For HH patients attending the LCH venesection clinic (i.e. patients who are having their iron overload proactively managed through phlebotomy) who have no co-morbidities, an echo is not warranted.

Clearly stated, if this patient type as described above does not have iron overload because it is being proactively managed, then the patient does not warrant an echo.

This hypothesis is tested using historical data gathered through a retrospective LCH venesection clinic patient record audit. It was hoped to prove that when HH patients with no other co-morbidities were treated through phlebotomy; iron overload was proactively managed and the results of the echoes performed would demonstrate the effectiveness of the treatment. Through this proactive treatment, the onset of cardiomyopathies in HH patients
with no other co-morbidities had been avoided. That is, the results would illustrate normal echoes with no significant cardiac abnormalities resulting from the initial HH iron overload diagnosis. Therefore, for this patient cohort, echoes were not warranted and the exponential increase in echo requests for this patient cohort would substantially decrease, alleviating demand on the echocardiography department.

### 6.1.5 Step 4: Secondary Research

Now that an initial hypothesis was created, the next step was to further investigate prevalent theories, concepts and practice in a broad literature review as detailed in chapters two, three, four and five.

A brief description on how the secondary research process was performed is as follows:

The literature reviewed covered published books, medical journals, medical articles and associated medical websites. Of particular interest were articles, experiments and clinical papers with similar investigations into echoes and treatment of Haemochromatosis patients worldwide.

From a topical viewpoint, HH was delved into; its definition, history, genotype and phenotype, its prevalence and penetrance in Europe and Ireland, its symptoms and its treatment. Two directly relevant observations come from this portion of secondary research. Firstly, that clinical evidence suggested only the C282Y polymorphism group is susceptible to iron overload disease (60-90%) although the risk is not well defined. All other polymorphisms have a very low risk of developing iron overload. However, in the LCH
venesection clinic patient cohort there were patients in the other polymorphism groups that had presented with iron overload.

Secondly, the phlebotomy treatment can easily and proactively manage Iron Overload in the body. In patients where no other co-morbidities are present, the result of this treatment is iron overload prevention. This intervention precludes the progression of the disease thereby avoiding tissue damage. Therefore, the patients of the LCH venesection clinic who present with no other co-morbidities should not warrant echoes to determine if there is adverse heart tissue damage.

An investigation of the blood, iron absorption, the role of hepcidin, iron overload, the effects of iron overload on the normal heart, the physiology and anatomy of the heart and echocardiography was undertaken. In this section evidence was presented which showed HH could lead to Iron Overload Cardiomyopathies.

Following the literature reviews the preliminary hypothesis was revisited and then moved into the methodology section of research design of the LCH venesection clinic patient record audit. Secondary research also continued in parallel to this activity and the hypothesis remained under review during this period.

6.1.6 Step 5: Hypothesis Review

In order to create a meaningful and succinct hypothesis for the study to test, the preliminary hypothesis was reviewed following the in-depth secondary research phase of this thesis. The secondary research validated the preliminary hypothesis, that is where there are no other co-morbidities, this patient cohort phlebotomy treatment is managing their iron overload disease resulting in a high probability that echoes on this cohort will be normal. Therefore, the echo
The report is adding little clinical value other than to confirm to clinicians the phlebotomy treatment is effective.

The aim of the hypothesis must now be rewritten to state the test scenario to be proven or disproven. The final hypothesis for this study was formulated as follows:

**HH patients with no co-morbidities attending the LCH venesection clinic for phlebotomy do not warrant an echocardiograph as their echo result will show no significant abnormalities relating to HH.**

The null hypothesis is the corollary statement:

**Hereditary Haemochromatosis patients with no co-morbidities attending the LCH venesection clinic for phlebotomy do warrant an echocardiograph as their echo result will have significant abnormalities relating to Hereditary Haemochromatosis.**

As stated earlier, proving or disproving this hypothesis will be tested using historical data gathered through a retrospective venesection patient record audit. In doing so it was hoped to prove that when HH patients with no other co-morbidities were treated through phlebotomy, iron overload was proactively managed and the results of the echoes performed would demonstrate the effectiveness of the treatment. That is, the results would illustrate normal echoes with no heart issues resulting from the iron overload diagnosis. Therefore, for this patient cohort, echoes are not warranted and the exponential increase in echo requests for this patient cohort would substantially decrease. This in turn would alleviate demand on the echocardiography department and decrease waiting list times.
6.1.7 Step 6: Research Design

A hypothesis is an educated prediction relating to an observed event. The observed event must be measurable so the hypothesis and the null hypothesis can be accepted or rejected.

In relation to this study the hypotheses have been described. Next, the study design will be elucidated; what measurements were directly relevant and why; and also from where and how the data was being collected, validated and analysed.

In general the study was a retrospective patient data audit and as such can best be described as observational research. The sample size was 878 patients and the method of sampling was purposive in nature and could particularly be described as ‘criterion sampling’ which involves searching for cases or individuals who meet certain criterion, for example, that they have had a certain disease,” (Palys; 2008). The sampling frame (the list from which the potential respondents are drawn) was the HH patient cohort attending the LCH venesection clinic for phlebotomy. The sampling procedure involved 100% of the patients recorded as attending this clinic.

6.1.8 How the Study was Designed

Selection of participants for this study and the methods of selection could determine the limit of generalisations that could be made from this study. As this was a purposive study to determine whether the LCH venesection clinic patient cohort need to be echoed or not, the selection of participants and method of selection was quite simple. The LCH venesection clinic has a list of patient attendees. This list had been compiled over the last number of years as new and transfer patients were added to the venesection clinic. The plan for this research study was to request access to this patient cohort list and then review the relevant medical data from the patient’s existing medical records. This data would be entered into an
excel spreadsheet database, anonymised and used as an analysis and test tool for the hypotheses.

“All research potentially raises ethical issues, and consent to proceed is required from relevant ethical committees before a research study can commence,” (Bowling: 2002). Therefore, the plan outlined above was submitted to the Health Service Executive Ethics Committee for approval of the retrospective patient data audit to ensure that all research was obtained ethically and anonymously. The Health Service Executive Ethics Committee signed approval along with the ethics Committee application form is shown in Appendix 2. Upon receipt of this approval to proceed data collation of the research data began.

An Excel database of 878 patients was created from the LCH venesection clinic list to allow the collection of the patient record data. The planned outcome of gathering this data was to test the hypotheses and present the conclusions to the Gastroenterology department so the LCH Guidelines could be reviewed and improved with this evidence based data.

The audit actions roadmap consisted of a venesection patient database focusing on nineteen primary information categories as shown in Table 6.1.

<table>
<thead>
<tr>
<th>Medical Record Number</th>
<th>Echo Date</th>
<th>Ferritin Level at Echo</th>
<th>Genetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Patient Echo Completed</td>
<td>Ferritin Level at Diagnosis</td>
<td>Laboratory Report</td>
</tr>
<tr>
<td>D.O.B.</td>
<td>Indication for Echo</td>
<td>Transferrin Saturation Level</td>
<td>Co-morbidities Evident</td>
</tr>
<tr>
<td>Gender</td>
<td>Indication for Echo H/H+</td>
<td>Hospital Transfer Data</td>
<td>Symptoms</td>
</tr>
<tr>
<td>Age at Diagnosis</td>
<td>Echo Findings</td>
<td>Venesected</td>
<td></td>
</tr>
</tbody>
</table>

Table 6.1 Categories of Data Collected for Analysis from the Medical Record Audit
The Medical Record Number, name and date of birth were anonymized for the purposes of data protection. The additional data categories were collected as they were central to the purpose of the research, that is: echo data, Ferritin and Transferrin levels, hospital transfer data, whether the patient had been venesected, the patient’s genetics relative to Haemochromatosis, Laboratory results, co-morbidities listed and symptoms. This data is believed to be central to the area under investigation as evidenced throughout the secondary research literary review.

The actual patient medical records were requested and over a period of six months (January to June 2015), reviewed and transferred the data onto an Excel database. This Excel database was kept on a HSE laptop which is encrypted to protect the patient data.

The fundamentals of each category were examined so that a grasp could be gained on how all the interlinked elements related to the literature review data, reports, comments and conclusions.

6.1.9 Inclusion and Exclusion Criteria

In total there were 878 patients on the LCH venesection list. However, there were limitations to this study as it was retrospective in design and limited to the records available. In some cases the physical patient records were not available. These patient records were each requested on three further occasions during the six-month research period, but were not returned for filing, and therefore not available for this retrospective study. This occurred in 45 instances (approximately 5%) and these records were excluded from the audit. Therefore, the patient records for data analysis totaled 833.

In other cases some of the main data on Echoes, Ferritin and Transferrin levels or Hospital Transfer data was not noted on the patient records. These were included in the 833 total as
some relevant data was recordable but not in 100% of all the 19 categories per patient record. The non-recordable data categories were excluded from some data analysis due to this status.

6.1.10 Methodology

During the previous chapters a number of objects and variables whose relationships could possibly have a causal nature with relation to HH and cardiomyopathies were identified. “Objects are regarded as variables that researchers may measure and/or control, and a variable is simply defined as a characteristic that may vary among the subjects or units of observation under study,” (Azavedo et al., 2011).

The hypotheses required an assessment of these relationships to ascertain if they are of a causal nature. It was believed that the inclusion of 833 (94.9%) of the 878 patient data records in the analysis is high enough to negate random errors compared to a population probability sample. The non-probability sample does not involve a random selection, therefore is representative of the venesection clinic HH population. However non-probability sampling cannot depend upon the rationale of probability theory and this is worth bearing in mind.

It was also believed that systematic errors could be present due to the human intervention of taking measurements and then recording them in a separate document or number of documents prior to the measurement recording being input on the patient data record. However, detailing the possible statistical error is beyond the focus of this retrospective patient record audit, and therefore is not taken into account. All measurements in the patient data records are taken to be true and accurate. To ensure the author’s recording was correct, a random selection of ten percent of all charts were reviewed to certify correct figures were transposed into the database.
The main areas of investigation regarding measurement parameters for HH as a summary of the literature reviews are: HH genotype and phenotype, diagnosis and disease progression, iron in the blood: transferrin saturation and serum ferritin levels, the effect of iron on the heart, cardiomyopathies and echocardiography.

6.1.11 Biomedical Data

The key ‘instrument’ for this retrospective patient record audit is the patient medical record. With exception of echocardiography which is central to this thesis, discussion of all other instruments used for the measurement of the recorded data in the patient medical record are beyond the scope of this audit.

The outcome of this audit would be to design a protocol for selection of patients for echo who have HH. The main purpose of the project was to investigate and gather data in the form of a patient record audit for the list of patients attending the venesection clinic at LCH.

From a customer (patient/doctor/HSE) viewpoint this research would hope to redefine prevailing hospital protocol to ensure that patients who required echoes received them in a timely manner where appropriate resources were applied cost effectively resulting in an efficient and effective service level achievement.

In conclusion, this dissertation was written from a learning outcome/research perspective and has retraced the steps that were necessary to understand HH, how it affects the physiology and myocardium of the heart and in turn what echo results would be expected and what echo results were actually reported. The data obtained was retrospective and spanned a number of years. This dissertation will also discuss the outcomes of the research and suggest some recommendations as a result of the data obtained.
6.1.12 Step 7: Patient Cohort Review

A total of 878 patients were on the LCH venesection clinic list. Some of these patient records were transferred from other hospitals and this fact was noted in the Excel database. In some cases full information on the patient was not transferred to the LCH patient record so not all patient record data could be included in the data analysis. Where patient data was not included, this fact is referred to in the relevant data analysis section.

For example, Fig. 6.2 illustrates the age range profile at diagnosis of the LCH venesection clinic patient cohort consisting of 833 patient records. There are five patients where the patient record data did not record the date of diagnosis; hence they are listed as ‘unknown’ for the purposes of analysis. 99 percent (828) of the patient records did give patient diagnosis date.

![Age Range at Diagnosis of 833 Patients](image)

**Fig. 6.2 Patient Age Range at Diagnosis of LCH Venesection Clinic Patient Cohort**
Chapter 7: Data Collection and Analysis

7.1 Introduction

The following section presents the main data analysis regarding the LCH Venesection Clinic retrospective patient record data review. Firstly, general data is presented regarding the patient cohort in relation to age and gender. Then specifics of genetic profile in relation to HH, Serum Ferritin Range at diagnosis and at time of echo is focused on and finally the echo results are reviewed. The next section will then present the main findings, with discussion, conclusions and recommendations of this retrospective patient record data and literature review in chapter 8 and chapter 9.

7.2 Data Analysis

As can be seen from Fig. 7.1 and in line with the general comment from the literature review, the LCH Venesection Clinic data analysis demonstrates the majority of patients (688) are diagnosed after the fourth decade in their lifecycle (83%). This finding concurs with the secondary research reviewed in chapter three, Fig .3.3 (Brandhagen, D.et al 2002), and as evidenced in a study which stated “Men with type 1 or type 4 hemochromatosis typically develop symptoms between the ages of 40 and 60, and women usually develop symptoms after menopause,” (US National Library of Medicine, 2016).

The record audit computation on age at diagnosis was made using the Date of Birth of the patient and the date of diagnosis per the data on the patient record. Then each patient record was categorised into age range groupings of: 16-30 years; 31-40 years; 41-50 years; 51-60
years and over 60 years. The date ranges were chosen with reference to the HH stages indicated in Chapter 3 on Hereditary Haemochromatosis.

**Fig. 7.1 Age at Diagnosis of LCH Venesection Clinic Patient Cohort**

**Fig. 7.2 Age at Diagnosis of LCH Venesection Clinic Patient Cohort by Gender**
In Fig 7.2 the overall the age profile demonstrated in the LCH Venesection Clinic data shows a similar age of diagnosis profile for males and females with the exception of the 31-40 age range, where it can be seen the male diagnosis cohort is four times (4:1) higher than the female diagnosis cohort where male patients outnumber females by only 2:1.

The genetic profile of the HH patients was also reviewed. As evidenced in chapter 3, in the 2010 EASL Clinical Practice Guidelines for HFE Hemochromatosis, a meta-analysis of 32 studies with 2,802 individuals with iron overload (clinically recognised haemochromatosis) is put forward demonstrating the prevalence of C282Y homozygosity (C282Y/C282Y) was 80.6% and compound heterozygosity (C282Y/H63D) was 5.3% and no carrier detail was mentioned.

In Fig. 7.3 the LCH Venesection Clinic data reports that 63 percent of patients were homozygous for C282Y (C282Y/C282Y), 9 percent were homozygous for H63D (H63D/H63D) and 26 percent were compound heterozygous C282Y/H63D. So our patient cohort demonstrates a lower incidence rate in the C282Y allele and a much increased prevalence of the C282Y/H63D allele.

![Genetic Profile of LCH Venesection Clinic Patient Cohort](image)

Fig. 7.3 Genetic Profile of LCH Venesection Clinic Patient Cohort
From a gender perspective the breakdown of the genetic prevalence is similar in the male and female profiles as shown in Fig 7.4.

**Fig. 7.4 Genetic Profile by Gender of LCH venesection Patient Cohort**

This profile can be seen in detail in Table 7.1. C282Y homozygous is the leading genetic profile with 59 percent of males and 69 percent of females carrying this gene. The next largest genetic profile are the C282Y/H63D heterozygous group with 27 and 23 percent retrospectively, then the H63D homozygous category with males at 11 and females at 7 percent.
Table 7.1 Genetic Profile by Gender of LCH venesection Patient Cohort

Serum Ferritin levels are an agreed method of reflecting iron in the body as evidenced in the literature review by Jacobs and Worwood, (1975) who proposed an assay of serum ferritin might lead to a “useful and convenient method of assessing the status of iron storage,” while Morrison et al. (2003) state that marked elevation of serum ferritin level has been associated with histologic evidence of iron deposition. The Serum Ferritin Range taken at patient diagnosis is shown in Fig. 7.5.

![Fig. 7.5 Serum Ferritin Range at Age of Diagnosis by Gender of LCH venesection clinic Patient Cohort](image)
The normal Serum Ferritin range for males and post-menopausal females is 300ng/ml and 200ng/ml for premenopausal females. In Fig. 7.5 the data from the LCH venesection clinic show that at age of diagnosis 61 percent of males (345) and 50 percent of females (137: <201ng/ml as the age of menopause cannot be determined) are above the nominal threshold for normal Serum Ferritin levels. This historic patient data is of interest as it can be compared to the post phlebotomy data in the next Fig. 7.6.

**Fig. 7.6 Serum Ferritin Range at Time of Echo by Gender of LCH Venesection Clinic Patient Cohort**

Fig. 7.6 demonstrates the positive effect of phlebotomy on Serum Ferritin levels in the patient cohort when compared to Fig. 7.5. We can now see that 85.6 percent of females (155) and 86.8 percent of males (323) had Serum Ferritin ranges at or below acceptable normal levels prior to echo. This demonstrates the effective and positive relationship venesection has on iron overload Hereditary Haemochromatosis. Compared to the Serum Ferritin Range levels
at diagnosis, where only 47 percent of females (127) had ranges at or below acceptable normal levels and 34 percent of males (193) had ranges at or below acceptable normal levels.

Finally, echo data related to both Serum Ferritin levels and co-morbidities in patients was reviewed. This is shown by gender breakdown in Fig. 7.7 (males) and Fig. 7.8 (females).

The literature review demonstrated that normal Serum Ferritin levels are an acknowledged measure of the level of iron in the body. These normal Serum Ferritin levels have been presented in the data analysis review above. Therefore, it is important to present the echo data analysis in relation to Serum Ferritin levels.

A total of 553 echoes were performed on the total patient cohort of 833, equating to 66.4 percent of the cohort. Of these echoes, 548 patients had recorded evidence of known pre-
echo Ferritin level data. This equates to 65.7 percent of the total patient cohort and 98.9 percent of all echoes performed.

Echoes performed on males with pre-echo Ferritin status in our patient cohort totaled 367. 86 percent of the male patient cohort (316) showed normal echo results. Even taking into account male patients with co-morbidities (107), 73 percent of these male patients (78) had normal echoes.

The expected echo results for patients with abnormal Serum Ferritin ranges would be for abnormal echoes in the majority of cases given the evidence that excessive iron is detrimental to heart health. However, the audit demonstrates echo results for this cohort were mainly normal (39) with only five abnormal echoes.

Our expectation of these 39 normal echoes would be the majority should also not have co-morbidities associated with them and this also holds true in 30 (76.9%) of these normal echoes.

Only 51 (13.9%) echoes demonstrated abnormal results. Of these, five patients with abnormal serum ferritin ranges had echo findings which were not specific to HH: one patient had a prior surgery (an Aortic valve replacement); one patient with had left ventricular hypertrophy; another patient had mild aortic stenosis; the fourth had a probable bicuspid aortic valve; and finally, one finding of mild aortic annular calcification. The patients mentioned here had increased ferritin at diagnosis and at the time of echo. Three of the five patients were C282Y/C282Y, one was H63D/H63D and one was C282Y/H63D.

Reviewing abnormal echoes in patients with normal ferritin at the time of echo and no co-morbidity (19), the main echo results showed mildly reduced left ventricular function and mild left ventricular hypertrophy. These findings are similar to those patients with abnormal
echoes, a co-morbidity and normal ferritin (27). There does not appear to be a direct correlation between the ferritin at diagnosis, the ferritin at the time of echo, the lack of co-morbidity and the echo results.

These 27 patients (normal serum ferritin at the time of echo with a co-morbidity with abnormal echo results), echo results were abnormal as would be expected. These results do seem to be related to the comorbidity and echo findings which were not specific to HH. 85% of this patient cohort had hypertension +/- another comorbidity i.e. ischemic heart disease, diabetes mellitus, atrial fibrillation, obesity and hypercholesterolemia. Most the patients exhibited more than one co-morbidity in this patient cohort. 96% (26/27) were >50 years of age. It is noteworthy to mention that in a normal population of adults >50 years of age in the Republic of Ireland the prevalence of hypertension is 64% (www.publichealth.ie.) This represents a higher than normal average but this was looking specifically at those patients with comorbidities.

As this patient audit review was based specifically on ferritin levels and their interaction with echo findings, it would be worth revisiting this data focusing on metabolic syndromes and HH in future research.
Echoes performed on females with pre-echo Ferritin status in our patient cohort totaled 180. 89 percent of the patient cohort (161) showed normal echo results. Even taking into account patients with co-morbidities (50), 82 percent of these patients (41) had normal echoes.

Patients with above normal Serum Ferritin ranges were mainly shown to evidence normal echoes (18) compared to 7 with abnormal echoes.

Taking a closer look at the data in Fig. 7.8, those patients with normal echoes, one or more comorbidities and normal serum ferritin range noted that 30/32 or 98% had hypertension. This was the main comorbidity. The majority (29/32) of this patient cohort were >50 years of age. In a normal population of adults >50 years in the Republic of Ireland the prevalence of hypertension is 64% (www.publichealth.ie), as previously mentioned.
Reviewing the data from the abnormal echo results. It would be expected that abnormally high ferritin may lead to abnormal echo results related to HH. Those five patients with normal serum ferritin, one or more comorbidities and an abnormal echo had the following echo findings: three patients had mild left ventricular hypertrophy and hypertension along with additional comorbidities (hyperlipidemia, obesity, hyperthyroidism and non-insulin dependent diabetes mellitus); one patient had mild to moderate left ventricular hypertrophy and associated hypertension; and finally, one patient had at least mild pulmonary hypertension and mild mitral regurgitation features associated with congestive heart failure. This particular patient had normal ferritin at diagnosis and normal ferritin at the time of the study. These findings are most likely not specific to HH given that all recorded serum ferritin levels have been within the normal range. All of the patients in this cohort were >60 years of age.

The four patients with abnormal serum ferritin, one or more comorbidities and an abnormal echo had the following echo findings: one patient demonstrated low normal function in association with hypertension and left bundle branch block; one patient exhibited mild left ventricular hypertrophy associated with hypertension and hypercholesterolemia; a third patient showed mild mitral stenosis with mild-moderate left ventricular dysfunction with atrial fibrillation and hypercholesterolemia; and finally, one patient had borderline left ventricular hypertrophy associated with hypertension and an abnormal liver profile. These echo findings were most likely co-related with the patient comorbidities. All of this patient cohort was >50 years old.

Finally, reviewing the patient cohort demonstrating abnormal echoes with no comorbidities and comparing the echo findings of those with normal and abnormal serum ferritin (10). The echo findings were not specifically related to HH. In fact, the echo findings for the most part
were unpredictable, with left ventricular hypertrophy as the main finding. In this cohort 90% of the patients were > 50 years of age.

Consolidating all echoes performed (male and female) where Serum Ferritin Level data was present and targeting patients with no co-morbidities only, we can see from the LCH Venesection Clinic data analysis that 92 percent of echoes (358) show normal results even when above normal Serum Ferritin levels are recorded, see Fig. 7.9.

![Bar chart showing echo results with normal and above normal serum ferritin levels](image)

**Fig. 7.9 Echoed Patients with Serum Ferritin and No Co-morbidities**

### 7.3 Presentation of Findings

The highlighted data analysis from the retrospective patient record audit of the LCH Venesection Clinic has been presented in the previous section. However, before launching
into this study’s findings and conclusions, a recap on this journey’s origin and the purpose of this retrospective study will follow.

In Chapter 1 and 2 and sections 6.1.1 and 6.1.2 of chapter 6, the reasons for commencing this study were presented. This journey’s origin was due to an exponential increase in the number of echoes being requested for HH patients from the LCH Venesection Clinic. The demonstrated data trend of increased requests showed a 1354% increase over a five year period from 2009 to 2014 (Fig. 1.1).

The objective aim of this study was to retrospectively investigate if an echo was warranted in the HH population at LCH Venesection Clinic and recommend to the Gastroenterology department whether the guiding principles of LCH should be retained or revisited if contrary to the conclusions.

The outcome of this study would possibly directly affect the future number of echocardiography requests from the HH venesection clinic and the echocardiography department’s ability to execute and report on all warranted requests in a clinically safe timeline whilst proactively managing the waiting list.

In preparation for the study, current guidelines from the British Society of Echocardiography (BSE), the European Association for the Study of the Liver (EASL) and The American College of Cardiology (ACC) were assessed. In conclusion, the BSE does not make any provisions for HH patients per se; the EASL Guidelines for HH refer specifically to symptomatic homozygous C282Y patients only, while noting that glycated hemoglobin (HbA1c), serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) should first be reviewed and if symptomatic, only then should echocardiography be performed; the ACC state those ‘at risk’ of IOC need echocardiography. Conversely, it was
suggested that those not ‘at risk’ of IOC do not require echocardiography, and as it has been shown through the literature review the incidence of IOC due to HH is extremely rare.

Extensive secondary research literature reviews were then carried out in Chapters 3 to 5. In Chapter 6, the storyboard of the study’s progression from its origins towards formulating a hypothesis to be tested was established. The hypotheses set out in step 5 (section 6.1.6) will now be revisited, then tested against the gathered data, findings summarised and discussion and conclusions presented.

The final hypothesis for this study was formulated as follows:

Hereditary Haemochromatosis patients with no co-morbidities attending the LCH venesection clinic for phlebotomy do not warrant an echocardiograph as their echo result will show no significant abnormalities relating to Hereditary Haemochromatosis.

The null hypothesis is the corollary statement:

Hereditary Haemochromatosis patients with no co-morbidities attending the LCH venesection clinic for phlebotomy do warrant an echocardiograph as their echo result will have significant abnormalities relating to Hereditary Haemochromatosis.

The first argument to disprove the null hypothesis and supporting the H1 hypothesis flows from Fig. 7.5 and 7.6 which demonstrate the positive effect of phlebotomy on HH iron overload patients. Comparing HH patients with normal Serum Ferritin Range levels at diagnosis, (60 percent of female and 34 percent of male patients) to the post phlebotomy/pre-echo levels (93 percent of female and 87 percent of male patients) reveals encouraging management of the iron overload phenomenon. These indications display the role of
phlebotomy in ensuring patients with HH preclude disease progression to the harmful iron overload cardiomyopathies.

Therefore, if the HH patients Serum Ferritin levels are being managed to ensure no iron overloading occurs, then the HH phlebotomy patients may no longer require repeat echoes as the very low incidence of IOC’s due to HH is now virtually non-existent.

Following on from this data analysis on proactive iron overload management, the focus moves to study the H1 hypotheses in essence: echo results for HH patients with no co-morbidities.

As can be seen in Fig. 7.7 and 7.8, the data to the left of the green demarcation lines on both male and female echo results show “normal echoes.” This status illustrates that of patients with recorded Serum Ferritin at time of echo (548 echoes), 87 percent (477) resulted in a “normal echo” report.

Coming closer now to the H1 hypothesis and reviewing only echo reports for those patients with no co-morbidities (390 patients), we can review Fig 7.7, 7.8 and 7.9. This patient sub-category indicates that 92 percent of echo reports (358) were “normal echoes.” This is true for both patients with Serum Ferritin levels in the normal (319) and above normal (39) ranges.

Therefore, the preliminary conclusion is the H1 Hypothesis is proven 92 percent of the time:

    Hereditary Haemochromatosis patients with no co-morbidities attending the LCH venesection clinic for phlebotomy do not warrant an echocardiograph as their echo result will show no significant abnormalities relating to Hereditary Haemochromatosis.
Chapter 8 : Discussion on LCH Hereditary Haemochromatosis Audit

8.1 Discussion of Findings

As stated in chapter 1, the initial investigation phase began with a review of the LCH guidelines in comparison with authorities in echocardiography (BSE), cardiology (ACC) and hepatology (EASL). No consistency of opinion from the three authority guidelines was evident. Therefore, this study’s hypothesis is of relevance in furthering practitioner understanding of the area through providing research of pertinent clinical data in order to inform and qualify current LCH guidelines on the usefulness of echo diagnostics in this HH venesection population.

Further to the overall literary research and the specific data analysis in chapter 7, the BSE assertion is to base the recommendation for performing echoes on clinical studies. It makes no provisions for HH patients per se and listed indications do not mention HH as an indication for Echocardiography. Therefore, the BSE is correct and an echo should not be performed in conditions for which there is no clinical study evidence and generally no accepted role, (WWW.bsecho.org).

Regarding the ACC algorithm for assessing patients who are ‘at risk’ of Iron Overload Cardiomyopathy (IOC), suggesting that an echo with full diastolic function assessment should be undertaken, the data analysis has shown the proactive phlebotomy management of HH patients is precluding iron overload and its potential to cause IOC. Additionally, “although cardiac failure is a recognised complication of severe iron overload, it is clinically
unusual except in juvenile HC,” (EASL; 2010). Therefore, this assessment will not be relevant to this particular patient cohort.

The EASL algorithm refers specifically to symptomatic homozygous C282Y patients only, and therefore their algorithm is not relevant to non C282Y patients in our study. So, for the C828Y patients only, the EASL state that if Serum Ferritin levels are normal – an annual follow up is proposed. This would indicate that patients already participating in the phlebotomy programme would not require a further echo. Also, where Serum Ferritin levels are increased an echo should only be warranted according to clinical features (EASL; 2010). Therefore, the clinical features, rather than the diagnosis of HH should influence the need for an echo. This conclusion is reiterated by the EASL stating that cardiac symptoms should be investigated by the cardiologist.

Reviewing the LCH venesection clinic data it was found that 67 percent of patients were male, 33 percent female (Fig 7.2). According to Genetrack, one of the largest DNA testing facilities in North America (www.hemochromatosisdna.com), this result is expected:

“Although both men and women can inherit the gene defect, men are more likely than women to be diagnosed with HH at a younger age. On average, men develop symptoms and are diagnosed between 30-50 years of age. For women the average age of diagnosis is about 50.”

Diagnosis of both males and females however, seems to be later in the Irish lifecycle in comparison to these US figures.

The age profile indicated that 83% of the LCH venesection clinic patients had their diagnosis after their fourth decade as predicated by Brandhagen et al. (2002). In the case of age at diagnosis profile Fig. 7.2, it was found that there was a significantly lower incidence of
diagnosis age in women in the 31–40 age category compared to men in this category. Again it was noted in commentary on this area throughout the literature review that this retrospective study concurs with this finding: “Men with type 1 or type 4 HH typically develop symptoms between the ages of 40 and 60, and women usually develop symptoms after menopause,” (US National Library of Medicine – (www.ghr.nlm.nih.gov/condition/hereditary-hemochromatosis)). These figures tie more closely to the LCH data analysis than the US data in the previous paragraph.

Looking at the HH genetic profile of the patients and comparing them to the literature reviewed in chapter 3, in particular to the Irish College of General Practitioners (2013), the overall prevalence in Ireland is gauged at over 93 percent for the homozygous C282Y allele. However, in general Europe demonstrates that 80.6 percent are normally homozygous for C282Y allele. In another large study in Newfoundland and Labrador (Kelley et al: 2014) between January 1999 and January 2009, totaling 4,138 individuals; 8.8% were C282Y homozygotes, 4.1% were H63D homozygotes, 6.4% were compound heterozygotes. Again, this confirms the prevalence of HH in northern European populations compared to other parts of the world as shown in Fig. 3.8.

Surprisingly the LCH Venesection Clinic data demonstrates a lower than expected incidence of C282Y homozygotes of 63% against the European average and nearly double the percentage for H63D homozygotes (9%) than the European average. This case could possibly be a subject for further research.

Taking into account the various stages of disease progression in HH patients as previously discussed in chapter 3, the patients attending LCH with no evidence of co-morbidities and ferritin levels within normal limits, fall into stage I (Fig. 3.2): “venesection treatment will allow tissue iron to be mobilized and iron stores will return to normal,”
(www.haemochromatosis-ir.com). This ties in with the recommendations as it demonstrates that where Serum Ferritin levels are being proactively managed, patients can lead a practically normal lifecycle with evidence that approximately 8 percent of patients with no other co-morbidities would have abnormal echoes.

To conclude this study, similarities and anomalies have been uncovered when relating the LCH HH venesection population to other similar studies. In general the data is aligned to that of previous studies. Overall the conclusion is a positive assertion that venesection is a successful, non-intrusive management tool for HH regardless of age of diagnosis, although serious complications are more apparent where HH is not diagnosed early. The use of echocardiography in this population could be decreased as the majority of the HH venesection patients were shown to have normal echoes.

The average cost of an echo in Ireland is approximately €230 based on a review of the VHI average costing. The opportunity cost of 319 echoes performed (with normal echo and no co-morbidity) is approximately €73,370. It is worth noting that echo service capacity remains constant irrespective of the indication on the request form. The price of an echo is not costed per se in LCH hospital on a case by case basis, however, the HSE are beginning to implement Activity Based Costing in certain sectors of the hospital. Therefore, removing this cohort of patients from the echocardiography waiting list would have an effect of reducing the number of echoes completed in the 2014 figures (Figure 1.1) from 189 to 8. From a pure cost perspective this could demonstrate a saving of €41,630 in 2014 alone and equates to roughly a month of echo man hours freed up.

The implication of adjusting the local echo policy extends beyond cost. There is more to value than just money. The 319 echoes performed equates to approximately 8 weeks of
workload at the LCH cardiology department and 319 patient administrations and follow-ups performed by other HSE staff.

As there was no prioritisation or vetting of echo requests in the past prioritisation on the echo out-patient list would have a positive knock on effect from the patient outcome viewpoint. There would be a reduction in the waiting list and patients would receive a more timely service possibly reducing the progression of a negative clinical outcome.

Subsequent to this audit the findings were presented to the venesection team and gastroenterologists in LCH. Both of these stakeholders agreed that subject to these findings they have decided to amend the local echo policy regarding the HH patient cohort. In future echoes will only be performed on patients with serum ferritin levels above 1,000 ng/ml.

This outcome will positively affect the echo waiting lists in LCH and result in improved and more focussed use of the limited resources of the Cardiology Department.
Chapter 9 : Conclusions and Recommendations from the LCH Hereditary Haemochromatosis Audit

9.1 Conclusion 1: The Study Hypothesis holds true and current LCH Guidelines need to be revised

9.2 Conclusion 2: The LCH venesection clinic age of diagnosis data generally follows previous study results

9.3 Conclusion 3: The Genetic Profile of the LCH venesection clinic data demonstrates lower than expected incidence of C282Y allele but higher than expected H63D allele incidence compared to previous study results

9.4 Conclusion 4: The LCH venesection clinic data demonstrates for patients with no co-morbidities, where Serum Ferritin levels are proactively managed through venesection, patients can lead a practically normal lifecycle

9.5 Conclusion 5: A significant and positive cost and patient service improvement could be achieved if the recommendations of this study were to be implemented
Recommendation 1 – Guiding Principles

The recommendation to the Gastroenterology Department that the guiding principles of LCH with regard to echocardiographic requests for HH patients with no co-morbidities who are attending the LCH Venesection clinic should be reappraised. This recommendation was accepted.

Recommendation 2 – HH Echo Patient Referral Pathway

The recommendation that there is no clinical reason for the LCH Venesection Clinic cohort containing patients with no co-morbidities and normal serum ferritin levels is to be put forward for echocardiography is valid. This recommendation was accepted.

Recommendation 3 – Further Research

The study recommendation is for the venesection clinic to instigate and maintain the database for this patient cohort. This recommendation was accepted.

An additional element noted during this audit would be to possibly research the patient comorbidities in more detail focussing on metabolic syndrome and its relationship with HH. This is for future discussion with the Gastroenterology Department.
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Appendices

Appendix 1 British Society of echocardiography - Indications for echocardiography

http://www.bsecho.org/indications-for-echocardiography/

British Society of echocardiography - Indications for echocardiography

Indications

1. Heart Murmurs

1.1 Indicated.

a. Murmur in the presence of cardiac or respiratory symptoms.

b. Murmur in an asymptomatic individual in whom clinical features or other investigation suggest structural heart disease.

1.2 Not Indicated.

a. Assessment of an innocent murmur diagnosed by a competent physician.

b. Unchanged murmur in an asymptomatic individual with previous normal echocardiogram.

2. Native Valvular Stenosis

2.1 Indicated.

a. Initial assessment of aetiology and severity, ventricular size and function

b. Repeat assessment of known stenosis with change in clinical status.

c. Periodic repeat assessment of asymptomatic individual with known severe stenosis for ventricular size and function

d. Repeat assessment of known stenosis in pregnancy

e. Assessment for pre-procedural decision-making for valvular intervention (e.g. suitability for balloon valvuloplasty)
f. Periodic repeat assessment of asymptomatic individual with moderate stenosis for valve severity, ventricular size and function.

2.2 Not Indicated.

a. Periodic repeat assessment of asymptomatic individual with haemodynamically-insignificant lesions, eg mitral annular calcification.

3. Native Valvular Regurgitation

3.1 Indicated.

a. Initial assessment of aetiology and severity, ventricular size and function

b. Initial assessment and risk stratification of individual with clinical signs of mitral valve prolapse

c. Repeat assessment in known regurgitation with change in clinical status.

d. Periodic repeat assessment of asymptomatic individual with known severe regurgitation for ventricular size and function

e. Periodic repeat assessment of asymptomatic individual with known mild or moderate regurgitation and ventricular dilatation or dysfunction

f. Periodic repeat assessment of asymptomatic individual with moderate MR

g. Repeat assessment of known regurgitation in pregnancy

h. Assessment for pre-procedural decision-making for valvular intervention (eg suitability for mitral valve repair - consider TOE and RT3D)

3.2 Not Indicated

a. Periodic repeat assessment of asymptomatic individuals with trivial or mild regurgitation and normal ventricular size and function.

b. Periodic repeat assessment of asymptomatic individuals with mitral valve prolapse and no or mild MR.

4. Prosthetic Valve Assessment

4.1 Indicated.

b. Late post-intervention re-evaluation for ventricular remodelling.

c. Repeat assessment of prosthetic valve with change in clinical status.

d. Repeat assessment of prosthetic valve with clinical findings suggestive of dysfunction

e. Repeat assessment of prosthetic valve following exposure to clinical risk of valve thrombosis

f. Periodic repeat assessment of asymptomatic individual with bioprosthetic valve (after 7 years for aortic bioprosthesis; after 5 years for mitral bioprosthesis) if intervention without symptoms would be undertaken.

4.2 Not Indicated

a. Periodic repeat assessment of asymptomatic individual with mechanical prosthesis

b. Repeat assessment of patients whose clinical status precludes therapeutic intervention

5. Infective Endocarditis

Note: In view of the possibility of both false-negative and false-positive studies, echocardiography should supplement but not replace clinical and microbiological diagnosis.

5.1 Indicated.

a. To characterise valvular lesions, haemodynamic consequences and ventricular response in a patient with clinically proven or suspected endocarditis.

b. Detection of high-risk complications, eg fistula, abscess, mass lesions.

c. TOE evaluation of patients with a high clinical suspicion following negative or equivocal TTE in native and prosthetic valves.

d. Persistent bacteraemia of unknown source, particularly in staphylococcal infection (consider TOE).

e. Baseline assessment of valve, ventricular size and function prior to discharge following completion of treatment for endocarditis.

5.2 Not Indicated.

a. Fever with no other suggestive features.

b. Periodic repeat assessment in a clinically stable patient with prior echocardiographic evaluation to assess response to therapy.
6. Ischaemic Heart Disease - Known or Suspected

6.1 Indicated.

a. Chest pain with haemodynamic instability

b. Murmur following acute or recent myocardial infarction.

c. Assessment of infarct size, presence of complications and baseline LV function following MI consider use of LV contrast echocardiography.

d. Evaluation of patients with non-diagnostic ECG and indeterminate laboratory markers if performed during or immediately after cardiac chest pain.

e. Evaluation of LV function to guide further therapy or assess effect of intervention, e.g. drug therapy, ICD implantation, CRT, patients scheduled to undergo coronary artery by-pass surgery.

g. Stress echocardiography to assess reversible ischaemia, myocardial viability and risk stratification.

6.2 Not Indicated.

a. Evaluation of non-cardiac chest pain

7. Cardiomyopathy

7.1 Indicated.

a. Clinical cardiomegaly

b. Clinical or radiographic signs of heart failure

c. Unexplained shortness of breath in the absence of clinical signs of heart failure if ECG/CXR abnormal

d. Persistent hypotension of unknown cause

e. Suspected cardiomyopathy based on abnormal examination, ECG, or family history in first degree relative

f. Baseline LV function and periodic review when using cardiotoxic drugs, eg herceptin

g. Repeat assessment in documented cardiomyopathy with change in clinical status

h. Repeat assessment in documented cardiomyopathy where result may change management or following procedures affecting function, eg cardiac resynchronisation, septal ablation.
7.2 Not Indicated.

a. Minor radiographic cardiomegaly in the absence of symptoms or signs of heart failure.
b. Routine repeat assessment in clinically stable patients in whom no change in management is contemplated.
c. Assessment of patients with oedema, normal venous pressure and no evidence of cardiac disease.

8. Pericardial Disease

8.1 Indicated.

a. Suspected pericarditis, pericardial effusion, tamponade or constriction
b. Suspected pericardial effusion or bleeding (post-surgery or trauma)
c. Periodic repeat assessment of moderate or large pericardial effusion
d. Repeat assessment of small pericardial effusion with change in clinical status
e. Echo-guided pericardiocentesis

8.2 Not Indicated.

a. Repeat assessment of small pericardial effusion without clinical change.
b. Pericardial friction rub following uncomplicated MI
c. Follow-up studies in patients with terminal illness whose management would not be affected by echocardiographic abnormalities

9. Cardiac Masses

9.1 Indicated

a. Embolic peripheral or neurological events suggesting intracardiac mass
b. Haemodynamic or auscultatory findings suggesting intracardiac mass
c. Periodic repeat assessment following removal of cardiac mass/tumour, eg myxoma
d. Known primary malignancies where echocardiographic surveillance for cardiac involvement forms part of the normal staging process, eg renal hypernephroma
9.2 Not Indicated.
a. Patients with terminal illness whose management would not be affected by echocardiographic abnormalities

10. Pulmonary Disease

10.1 Indicated.
a. Lung disease with clinical suspicion of cardiac involvement (cor pulmonale)
b. Suspected or established pulmonary hypertension
c. Suspected or established pulmonary embolism to inform a decision regarding thrombolysis
d. Evaluation for surgical procedures for advanced lung disease including transplantation
e. Repeat assessment of pulmonary artery pressure to evaluate response to treatment for pulmonary hypertension (consider saline contrast enhancement)
f. To distinguish cardiac from non-cardiac causes of dyspnoea when the results of clinical and other diagnostic testing are ambiguous
g. Patients with known chronic lung disease and unexplained desaturation (consider saline contrast echocardiography)

10.2 Not Indicated.
a. Lung disease with no clinical suspicion of cardiac involvement

11. Neurological Disease

11.1 Indicated.
a. Acute interruption of blood flow to major peripheral or visceral artery
b. Unexplained stroke or TIA without evidence of prior cerebrovascular disease or without significant risk factors for other cause (consider saline contrast echocardiography by TTE or TOE). The importance of a PFO if found when performing contrast studies may depend on the patient's age and may therefore only be appropriate in those under 55.
c. Patients for whom a therapeutic decision will depend on outcome of echocardiography, eg anticoagulation
d. Assessment of neuromuscular diseases associated with cardiac manifestations, eg muscular dystrophies, mitochondrial myopathies and periodic paralyses
e. Hemiplegic migraine (saline contrast study)

11.2 Not Indicated.

a. Patients in whom echocardiography will not affect decision to commence anticoagulation (eg patients in AF with cerebrovascular event and no suspicion of structural heart disease)

12. Arrhythmia, Palpitations and Syncope

12.1 Indicated.

a. Clinical suspicion of structural heart disease in proven arrhythmia

b. Assessment of ventricular function for primary prevention of sudden cardiac death (SCD) post-MI

c. Assessment of ventricular function for secondary prevention of SCD in VT

d. Evaluation of LV function prior to anti-arrhythmic medication

e. Syncope in a patient with clinically suspected heart disease

f. Exertional syncope

g. Syncope in a patient with high-risk occupation, eg pilot, bus driver

h. Assessment of patients without clinical suspicion of structural heart disease who have an arrhythmia commonly associated with structural heart disease

i. Guidance of catheter placement during radiofrequency ablation (consider TOE or intracardiac echo)

j. Post-operative evaluation of patients following RF ablation and surgical procedures in the absence of complications

12.2 Not indicated

a. Palpitations without proof of arrhythmia or clinical suspicion of structural heart disease

b. Isolated premature ventricular contractions in absence of clinical suspicion of structural heart disease.

c. Classic neurocardiogenic syncope
13. Echocardiography Before Cardioversion

13.1 Indicated

a. Guidance for decision to attempt cardioversion, eg LV function, MV disease

b. Patients requiring cardioversion with AF >48hours duration not adequately anticoagulated (TOE)

c. Repeat assessment of documented appendage thrombus (TOE)

d. Repeat assessment following embolic event at previous cardioversion (TOE)

e. Patients with AF less than 48hrs duration and clinical suspicion of structural heart disease not adequately anticoagulated (consider TOE).

f. Patients undergoing cardioversion for atrial flutter

13.2 Not indicated

a. Patients requiring emergency cardioversion

b. Patients on long-term anti-coagulation at therapeutic level with no clinical suspicion of structural heart disease

c. Patients on long-term anti-coagulation at therapeutic level with structural heart disease but no recent clinical change

14. Hypertension

14.1 Indicated

a. Suspected LV dysfunction

b. Evaluation of LVH and LV remodelling where this will alter management

c. Evaluation of clinically suspected aortic coarctation.

14.2 Not indicated

a. Routine assessment

b. Repeat assessment of LV function in asymptomatic patients

c. Repeat assessment for LV mass regression
15. Aortic and Major Arterial Disease

15.1 Indicated

a. Suspected aortic dissection: diagnosis, location, extent (TOE)

b. Assessment of aortic aneurysm and aortic dilatation: diagnosis, location, extent (consider TOE)

c. Suspected aortic rupture and intramural haematoma

d. Periodic repeat assessment of aortic root and ascending aortic dilatation

e. Assessment of suspected or proven connective tissue disorder in which aortic pathology may be a feature, eg Marfan’s

f. Repeat assessment of prior surgical repair of aorta

15.2 Not indicated

None relevant

16. Pre-Operative Echocardiography for Elective and Semi-urgent Surgery

16.1 Indicated

a. Documented ischaemic heart disease with reduced functional capacity (<4 METS)

b. Unexplained shortness of breath in the absence of clinical signs of heart failure if ECG and/or CXR abnormal

c. Murmur in the presence of cardiac or respiratory symptoms

d. Murmur in an asymptomatic individual in whom clinical features or other investigation suggest severe structural heart disease.

16.2 Not indicated

a. Repeat assessment of previous echocardiogram with no intervening change in clinical status

b. Routine pre-operative echocardiography

In-Patient Triage

Appropriate triage categorization is dependent on accurate information being given in the request form. It is recognized that failure to provide adequate information may lead to delay.
The triage categories are:

**Category 1 (Emergency)**

Echocardiography to be done immediately

8.1a Likely acute pericardial tamponade (following interventional procedure including intracardiac catheter or pacing manipulation)

10.1c Likely acute (massive) pulmonary embolism to inform a decision regarding thrombolysis

**Category 2**

Result likely to change immediate patient management and to be done <24 hours. Priority within that time to be discussed (may be emergency)

5.1b Detection of high-risk complications of infective endocarditis where patient is haemodynamically unstable.

6.1b Murmur following acute or recent myocardial infarction where papillary muscle rupture or ventricular septal rupture suspected.

7.1d Persistent hypotension of unknown cause where patient haemodynamically unstable and not responding to intensive care.

8.1a Suspected pericardial tamponade.

8.1b Suspected pericardial effusion or bleeding (including after serious chest trauma).

15.1a Suspected aortic dissection (including following possible deceleration injury)

**Category 3**

Echocardiography indicated but may not change immediate management. Echocardiography should be done as an in-patient if possible. If resources do not allow this, it may be performed as an outpatient but should be discussed with the referring clinicians.

All other indications for echocardiography.
Appendix 2 Scan of signed off Ethics Approval
27th November 2014

Ms Lorna Doran
Senior Cardiac Technician
HSE Louth County Hospital
Dublin Road
Dundalk
Co Louth

Re: Research Study Proposal:
"Retrospective Echocardiographic analysis of patients with Hereditary Haemochromatosis (HH) to confirm if an Echo is warranted in this patient population based on current Guidelines, focusing particularly on Ventricular dimensions, LV systolic and LV diastolic function"

Dear Ms Doran

I would like to advise you that the following documentation was reviewed by the HSE North East Area Research Ethics Committee on Thursday 20th November 2014 in the Board Room, HSE Dublin North East Office, Bective Street, Kells, Co. Meath.

Protocol Title: Retrospective Echocardiographic analysis of patients with Hereditary Haemochromatosis (HH) to confirm if an Echo is warranted in this patient population based on current Guidelines, focusing particularly on Ventricular dimensions, LV systolic and LV diastolic function

Application Date: 23rd October 2014

Documentation Reviewed:
- Completed Application Form
- Local Committee Declaration & Signatory Page signed by Academic Research Supervisor by Prof Pat Goodman, Course Director Clinical Physiology,
- Principal Investigator 2 paged CV
- Site/Service Specific Assessment Form for Research approved and signed by Ms Louise O'Hare, Hospital Administrator, Louth County Hospital, Dundalk, Co Louth and PP for Dr John Keoghane, Consultant Gastroenterologist, Our Lady of Lourdes Hospital / Louth County Hospital

Applicant Name: Ms Lorna Doran
Applicant Title: Senior Cardiac Technician
Decision Date: 20th November 2014
Members Present: Dr Brendan MacMahon (Chair)
Ms Margaret Scott
The Committee has recommended a favourable opinion for the above research based on the application form and supporting documentation.

This favourable opinion is given provided that you comply with the conditions set out in the attached document.

If during the course of the research project, amendments or alterations to the proposed research are required, approval must again be sought from this Committee.

Yours sincerely,

Dr Brendan MacMahon
Chairperson
HSE North East Area -
Research Ethics Committee

Copied to/ Prof Pat Goodman, Course Director Clinical Physiology, DIT, Kevin Street
Ms Louise O’Hare, Hospital Administrator, Louth County Hospital, Dundalk, Co Louth
Dr John Keohane, Consultant Gastroenterologist, Our Lady of Lourdes
Hospital/ Louth County Hospital
HSE North East Area Research Ethics Committee  
HSE Dublin North East, Bective Street, Kelts, Co. Meath

List of sites with favourable opinion

Research Identification

<table>
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<th>Title of Research:</th>
<th>Retrospective Echocardiographic analysis of patients with Hereditary Haemochromatosis (HH) to confirm if an Echo is warranted in this patient population based on current Guidelines, focusing particularly on Ventricular dimensions, LV systolic and LV diastolic function</th>
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<td>Submission date:</td>
<td>23rd October 2014</td>
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LIST OF CONDITIONS

This study was given a favourable opinion on Thursday 20th November 2014 subject to the following:

- Approval was given for the study to commence subject to confirmation that Consultants whose patient’s records are the subject matter are informed and approve of access to those records for the purpose of this study.

The Study was approved subject to the above requirement.

Please forward response by email to eimhear.dowling@hse.ie for my review by the 10th December 2014.

Please note that final approval to commence your study cannot be given until such time as the above requirements are met.

The favourable opinion is extended to each of the sites listed below.

<table>
<thead>
<tr>
<th>Applicant</th>
<th>Site</th>
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<tbody>
<tr>
<td>Ms Lorna Doran</td>
<td>Louth County Hospital, Dundalk</td>
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</table>

Signed: ____________________________  
Chair of Committee

Date: 27th November 2014
Appendix 3 Classification of Iron Overload Syndromes


Classification of Iron Overload Syndromes

Hereditary Hemochromatosis

*HFE*-related
- C282Y/C282Y
- C282Y/H63D
- Other *HFE* mutations

Non-*HFE*-related
- Hemojuvelin (*HJV*)
- Transferrin receptor-2 (*TfR2*)
- Ferroportin (*SLC40A1*)
- Hepcidin (*HAMP*)
- African iron overload

Secondary Iron Overload

Iron-loading anemias
- Thalassemia major
- Sideroblastic
- Chronic hemolytic anemia
- Aplastic anemia
- Pyruvate kinase deficiency
- Pyridoxine-responsive anemia

Parenteral iron overload
- Red blood cell transfusions
- Iron–dextran injections
- Long-term hemodialysis

Chronic liver disease
- Porphyria cutanea tarda
- Hepatitis C
- Hepatitis B
- Alcoholic liver disease
- Nonalcoholic fatty liver disease
- Following portocaval shunt

Dysmetabolic iron overload syndrome

Miscellaneous
- Neonatal iron overload
- Aceruloplasminemia
- Congenital atransferrinemia

<table>
<thead>
<tr>
<th>Table 1: Major clinical syndromes related to iron overload (1-4)</th>
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<tbody>
<tr>
<td>1. Primary</td>
</tr>
<tr>
<td>a. HH - gene HFE (Type 1)</td>
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<tr>
<td>b. HH - Juvenile (Type 2)</td>
</tr>
<tr>
<td>Hemojuvelin (Type 2A)</td>
</tr>
<tr>
<td>Hepcidin (Type 2B)</td>
</tr>
<tr>
<td>c. HH - transferrin receptor 2 (Type 3)</td>
</tr>
<tr>
<td>d. HH - ferrophin gene (Type 4)</td>
</tr>
<tr>
<td>e. Other types:</td>
</tr>
<tr>
<td>HH - heavy-chain ferritin gene</td>
</tr>
<tr>
<td>Aceruloplasminemia</td>
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<tr>
<td>DMT1 mutation (neonatal HH)</td>
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<tr>
<td>Atransferrinemia</td>
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<td>de Friedrich Ataxia</td>
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<td>2. Secondary</td>
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<td>2.1 Transfusional</td>
</tr>
<tr>
<td>a. Chronic hemolytic anemia (thalassemias, sickle cell disease)</td>
</tr>
<tr>
<td>b. Myelodisplastic syndrome</td>
</tr>
<tr>
<td>c. Aplastic anemia</td>
</tr>
<tr>
<td>d. Fanconi anemia</td>
</tr>
<tr>
<td>e. Blackfan Diamond anemia</td>
</tr>
<tr>
<td>2.2 Non-transfusional</td>
</tr>
<tr>
<td>f. Chronic hepatic disease</td>
</tr>
<tr>
<td>- viral hepatitis (virus B, C)</td>
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<td>- alcohol-induced hepatitis</td>
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<tr>
<td>- metabolic syndrome</td>
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<tr>
<td>- non-alcoholic steatohepatitis</td>
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<tr>
<td>g. Late cutaneous porphyria</td>
</tr>
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<td>h. Portacaval shunt</td>
</tr>
<tr>
<td>i. African iron overload</td>
</tr>
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# Appendix 4 Prevalence of the common HFE polymorphisms

Prevalence of the common HFE polymorphisms C282Y and H63D in the general population.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Ref.</th>
<th>Country – Population</th>
<th>Individuals screened</th>
<th>Allele frequency for c.845 C &gt; A (Y282)</th>
<th>c.187 C &gt; G (D63)</th>
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<td>Greece</td>
<td>196</td>
<td>0.013</td>
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<td>7620</td>
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<td>Allele frequency for</td>
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<td>Steinberg et al. (2001)</td>
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<td>[17]</td>
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<td>30,672</td>
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<td>Jones et al. (2002)</td>
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<td>Candore et al. (2002)</td>
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<td>Salvioni et al. (2003)</td>
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<td>Sanchez et al. (2003)</td>
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<td>Mariani et al. (2003)</td>
<td>[31]</td>
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<td>1132</td>
<td>0.032</td>
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<td>Altes et al. (2004)</td>
<td>[32]</td>
<td>Spain – Catalonia</td>
<td>1043</td>
<td>0.0282838</td>
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<td>Adams et al. (2005)</td>
<td>[33]</td>
<td>USA – whites</td>
<td>44,082</td>
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<tr>
<td>Barry et al. (2005)</td>
<td>[34]</td>
<td>USA – non-Hispanic whites</td>
<td>3532</td>
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<td>Meier et al. (2005)</td>
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<td>Germany</td>
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<td>Matas et al. (2006)</td>
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<td>0.00784314</td>
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<td>Allele frequency for C282Y</td>
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<td>Hoppe et al. (2006)</td>
<td>[38]</td>
<td>USA – non-Hispanic whites</td>
<td>991</td>
<td>0.05499495, 0.134207871</td>
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<tr>
<td>Aranda et al. (2007)</td>
<td>[39]</td>
<td>Spain – Northeastern</td>
<td>812</td>
<td>0.03140394, 0.219211823</td>
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<tr>
<td>Terzic et al. (2006)</td>
<td>[40]</td>
<td>Bosnia and Herzegovina</td>
<td>200</td>
<td>0.0225, 0.115</td>
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<tr>
<td>Floreani et al. (2007)</td>
<td>[41]</td>
<td>Italy – Central</td>
<td>502</td>
<td>0.0189243, 0.148406375</td>
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<tr>
<td>Raszeja-Wyszomirski et al. (2008)</td>
<td>[42]</td>
<td>Poland – Northwestern</td>
<td>1517</td>
<td>0.04416612, 0.154251813</td>
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</table>

From this allelic frequency for C282Y, a genotype frequency of 0.38% or 1 in 260 for C282Y homozygosity can be calculated from the Hardy–Weinberg equation. The reported frequency of C282Y homozygosity is 0.41%, which is significantly higher than the expected frequency. This probably reflects a publication or ascertainment bias.

Significant variations in frequencies of the C282Y allele between different geographic regions across Europe have been reported with frequencies ranging from 12.5% in Ireland to 0% in Southern Europe (Fig. 1).
Appendix 5 Prevalence of HH and percentage of study subjects with lab Evidence of the disorder


Prevalence of C282Y homozygosity and C282Y/H63D compound heterozygosity in clinically recognized hemochromatosis.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Ref.</th>
<th>Study population</th>
<th>Prevalence of HLA/HFE among clinical hemochromatosis cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. of cases</td>
</tr>
<tr>
<td>Feder et al... (1996)</td>
<td>[1]</td>
<td>USA – Multicenter</td>
<td>187</td>
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<tr>
<td>Jazwinska et al... (1996)</td>
<td>[43]</td>
<td>Australia</td>
<td>112</td>
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<tr>
<td>Jouanolle et al... (1996)</td>
<td>[44]</td>
<td>France</td>
<td>65</td>
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<tr>
<td>Beutler et al... (1996)</td>
<td>[45]</td>
<td>USA – European origin</td>
<td>147</td>
</tr>
<tr>
<td>Authors</td>
<td>Ref.</td>
<td>Study population</td>
<td>Prevalence of HLA/HFE among clinical hemochromatosis cases</td>
</tr>
<tr>
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<td>------</td>
<td>------------------</td>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>Carella et al., (1997)</td>
<td>[47]</td>
<td>Italy – Northern</td>
<td>75 48 5</td>
</tr>
<tr>
<td>Cardoso et al., (1998)</td>
<td>[51]</td>
<td>Sweden</td>
<td>87 80 3 1</td>
</tr>
<tr>
<td>Sanchez et al., (1998)</td>
<td>[52]</td>
<td>Spain</td>
<td>31 27 2 1</td>
</tr>
<tr>
<td>Ryan et al., (1998)</td>
<td>[53]</td>
<td>Ireland</td>
<td>60 56 1 2</td>
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<tr>
<td>Nielsen et al., (1998)</td>
<td>[54]</td>
<td>Germany – Northern</td>
<td>92 87 4</td>
</tr>
<tr>
<td>Murphy et al., (1998)</td>
<td>[55]</td>
<td>Ireland</td>
<td>30 27</td>
</tr>
<tr>
<td>Mura et al., (1999)</td>
<td>[56]</td>
<td>France – Brittany</td>
<td>711 570 40 35</td>
</tr>
<tr>
<td>Bacon et al., (1999)</td>
<td>[58]</td>
<td>USA</td>
<td>66 60 2</td>
</tr>
<tr>
<td>Authors</td>
<td>Ref.</td>
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<td>Prevalence of HLA/HFE among clinical hemochromatosis cases</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------</td>
<td>------------------</td>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>Brandhagen et al., (2000)</td>
<td>[60]</td>
<td>USA – Liver transplant recipients</td>
<td>5 4</td>
</tr>
<tr>
<td>Rivard et al., (2000)</td>
<td>[60]</td>
<td>Canada – Quebec</td>
<td>32 14 3 8</td>
</tr>
<tr>
<td>Brandhagen et al., (2000)</td>
<td>[63]</td>
<td>USA</td>
<td>82 70 2</td>
</tr>
<tr>
<td>Sham et al., (2000)</td>
<td>[64]</td>
<td>USA – Minnesota</td>
<td>123 74 15 6</td>
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<td>Van Vlierberghe et al., (2000)</td>
<td>[65]</td>
<td>Belgium – Flemish</td>
<td>49 46 2 1</td>
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<tr>
<td>Bell et al., (2000)</td>
<td>[66]</td>
<td>Norway</td>
<td>120 92 3</td>
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<tr>
<td>Hellerbrand et al., (2001)</td>
<td>[67]</td>
<td>Germany – Southern Spain</td>
<td>36 26 3 2</td>
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<tr>
<td>de Juan et al., (2001)</td>
<td>[68]</td>
<td>Basque population</td>
<td>35 20 4 2</td>
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</table>
The authors concluded that (1) future studies to discover modifier genes that affect phenotypic expression in C282Y hemochromatosis should help identify patients who are at greatest risk of developing iron overload who may benefit from continued monitoring of iron status, and (2) although genetic testing is well-accepted and associated with minimal risk of discrimination, generalized population screening in a primary care population as performed in the HEIRS study is not recommended.

In a substudy of Caucasian participants in the HEIRS study, Adams et al (2013) assessed the prevalence of HFE mutations in patients who had elevated serum ferritin levels less than 1000 µg/L (300-1000 µg/L for men, 200-1000 µg/L for women).(20) Among 3359 men and 2416 women, prevalence of potential iron-loading HFE genotypes (defined as C282Y homozygote, C282Y/H63D compound heterozygote, or H63D homozygote) was 10% and 12% in men and women, respectively. Prevalence of C282Y homozygosity was 2% and 4% among men and women, respectively. Likelihood of C282Y homozygosity increased with increasing serum ferritin levels, from 0.3% to 16% in men, and from 0.3% to 30% in women. Posttest likelihood ratios (likelihood of C282Y homozygosity given a positive test result) exceeded 1 at serum ferritin levels of 500 µg/L or more for men and at levels greater than 300 µg/L for women. In Caucasian subjects with mild hyperferritinemia, causes of elevated serum ferritin level other than C282Y or H63D HFE mutations (eg, liver disease, diabetes) were more likely.
Appendix 6 A BSE Guideline Protocol for the Echocardiographic assessment of Diastolic Dysfunction

NEW GUIDELINES
A Guideline Protocol for the Echocardiographic assessment of Diastolic Dysfunction (Published November 2013)

Echocardiography plays a central role in the non-invasive evaluation of diastole and should be interpreted in the clinical context.

Multiple echocardiographic measurements have been proposed to assess diastolic function but no single parameter should be used in isolation. This document gives recommendations for the image and analysis dataset required for the assessment of diastolic dysfunction (DD) using established indices acquired as part of the minimum dataset. Due to the variable sensitivity and specificity of the available parameters in different clinical settings, contradictory data can occur and in a proportion of patients a final diagnosis may not be achieved. In these situations the conventional echo data should be supplemented with information from other forms of assessment including haemodynamic measurement.

In this document, the parameters assessed are first set out systematically, with the key measurements highlighted in bold. For ease of reference they are also listed below. A simplified flow chart follows to assist in diastolic dysfunction grading.

Appendix 1 summarises normal values. Appendix 2 provides recommendations on assessing diastolic function in specific clinical situations.

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Dr Rick Steeds, Chair
Dr Richard Jones
Dr Prathap Kanagala
Dr Guy Lloyd
Dr Daniel Knight
Dr Kevin O’Gallagher
Dr David Oxborough
Dr Bushra Rana
Dr Liam Ring
Julie Sandoval
Gill Wharton
Dr Richard Wheeler

Abbreviations:
NOTE: key parameters are highlighted in bold. The remaining parameters are useful adjuncts when the diagnosis of diastolic dysfunction severity remains unclear.

- **E Vmax**: Mitral valve early filling on PW Doppler (m/s)
- **A Vmax**: Mitral valve atrial filling (m/s)
- **A dur**: Duration of atrial filling wave on PW Doppler (ms)
- **E/A ratio**: Ratio of E Vmax/A Vmax
- **DT**: Deceleration time (ms)
- **PV s**: Pulmonary vein systolic wave peak velocity (m/s)
- **PV d**: Pulmonary vein diastolic wave peak velocity (m/s)
- **PV s/d**: Ratio of pulmonary vein peak systolic velocity/peak diastolic velocity
- **PV a dur**: Duration of atrial reversal from PW Doppler of pulmonary vein flow (ms)
- **LAI**: Left atrial volume indexed to body surface area (mls/m2)
- **e’**: Velocity of early myocardial relaxation measured on tissue Doppler imaging (cm/s)
- **E/e’**: Ratio of MV E Vmax/ tissue Doppler early myocardial relaxation velocity
- **Mitral Vp**: Propagation velocity of early filling wave into the LV (cm/s)
<table>
<thead>
<tr>
<th>VIEW</th>
<th>Modality</th>
<th>Measurements</th>
<th>Explanatory note for ARVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 4C</td>
<td>PW Doppler</td>
<td>E Vmax, A Vmax, E/A ratio</td>
<td>Sample volume is placed at the level of mitral leaflets tips (colour flow can be helpful for optimal alignment, particularly when LV is dilated)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DT</td>
<td>Optimise spectral gain/wall filters to ensure clear crisp signal of onset and cessation of LV inflow</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A dur</td>
<td>Measurements are obtained over 3 cardiac cycles at end expiration</td>
</tr>
<tr>
<td></td>
<td>PW Doppler with Valsalva</td>
<td>Change in Mitral E/A ratio</td>
<td>See appendix 1 for normal values</td>
</tr>
<tr>
<td></td>
<td></td>
<td>from baseline</td>
<td></td>
</tr>
</tbody>
</table>

- **Normal**:

- **Grade 1**: Decrease in 20cm/s in E wave velocity generally indicates a good Valsalva technique

- **Grade 2**: Decrease in mitral E/A ratio of ≥50% is highly specific of raised LV filling pressure.

- **Grade 3 before Valsalva**: Useful when differentiating grade 2 from normal.
PW Doppler

**PVs**
**PQd**
**s/d ratio**

**a-dur**

Superior angulation of the transducer in the 4C view and colour flow is often required to locate the right upper pulmonary vein (seen close to atrial septum).

Sample volume is placed >0.5cm into the pulmonary vein.

Wall filter settings should be lowered with a faster sweep speed (50-100mm/s) to optimise recording; aim to include clear visualisation of atrial reversal velocity waveform.

Measurements are obtained over 3 cardiac cycles at end expiration.

If there are 3 systolic peaks (S1 and S2), peak S2 should be used to compute s/d ratio.

See appendix 1 for normal values.

Calculate: a-dur - A-dur

a-dur - A-dur of more than 30ms indicates raised IV filling pressure.
| A 4C | Tissue Doppler imaging (TDI) | e' | Velocities are recorded using PWTDI and not colour coded TDI |
| A 4C | Colour M-Mode | Calculate : E/e' ratio (Vp) | Sample volume is placed at or within 1cm of the insertion site of mitral valve leaflets. Optimise the velocity scale and baseline to demonstrate full signal. Gain settings should be adjusted to display high amplitude annular velocities. Measurements are obtained over 3 cardiac cycles at end expiration. e' is unreliable in the presence of mitral annular calcification, mitral prosthetic valves and annuloplasty rings and severe mitral valve disease. See appendix 1 for normal values. e' - average from 2 sites (lateral and septal) is used for the ratio. |
| | CW Doppler | TR V max | Acquisition is performed in the 4c view with colour flow imaging (narrow colour sector) across the mitral valve and an M-mode line placed through the centre of the LV inflow blood column (MV to LV apex). Nyquist limit is adjusted to display the central highest velocity jet as blue. Flow propagation velocity (Vp) is measured as the slope of the first aliasing velocity measured from mitral valve plane to 4 cm distally in to the LV cavity. Mitral E/Vp ratio can be used to predict LA pressure. E/Vp > 2.5 indicates elevated LA pressure (>15mmHg). In the absence of lung or mitral valve disease, raised PA pressure may indicate DD. |
| A 4C & A 2C | 2D | LA volume | Average volume measured at ventricular end systole (LA largest) using Modified Simpsons or Area Length method and indexed to BSA. See minimum dataset and chamber quantification guidelines. |

| Normal Vp |
Appendix 2

In certain clinical situations, conventional echo indices cannot be readily applied to assess diastolic dysfunction. The following section provides recommendations on assessing diastolic function in this group of patients. In these patients, grading of DD is not always possible and the aim is to estimate the filling pressures as a marker of diastolic dysfunction.

a. Left ventricular hypertrophy: In patients with heart failure symptoms and normal EF, evidence of concentric remodelling or raised LV mass index is itself indicative of diastolic dysfunction. In this group of patients, assessment of other markers of diastolic dysfunction does not provide additional diagnostic information.
b. Sinus tachycardia: E/A fusion occurs rendering E/A ratio and deceleration time unreliable in assessing DD. E/e’ ratio using fused peak mitral inflow velocity and peak fused mitral annular velocity can still be used to predict LV filling pressures in this situation.
c. Atrial Fibrillation: Loss of atrial contraction, variable cycle length and the frequent occurrence of atrial dilatation limit the usefulness of conventional indices in the assessment of DD. DT and E/e’ ratio averaged over 5-10 cardiac cycles (recorded from cycle lengths equivalent to a heart rate between 60-80 beats/minute) can be used to assess LV filling pressures in this group.
d. Constrictive pericarditis: Constrictive pericarditis can present with heart failure symptoms and restrictive filling pattern (Grade III) in the absence of diastolic dysfunction. Normal or increased e’ velocity can differentiate this condition from DD.
e. Mitral valve disease: Mitral E Vmax and PVs are affected by significant primary MR. A dur- A dur is the strongest predictor of LV filling pressure in this situation.
f. Systolic dysfunction: Grading of DD and estimation of filling pressures provide additional prognostic information in patients with established systolic dysfunction. Mitral inflow pattern (E/A ratio and DT) alone can be used to estimate filling pressure in this population and no further evaluation is necessary except in borderline cases. Accordingly E/A ratio < 1 in this population often indicates normal filling pressures and E/A ratio of 1-2 or > 2 strongly suggest raised pressures.

References:
Appendix 7  Mitral Inflow Pulsed Wave Doppler Profiles

<table>
<thead>
<tr>
<th>Mitral inflow</th>
<th>Mitral annulus velocity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Relaxation abnormality</td>
</tr>
<tr>
<td></td>
<td>Pseudo-normalization</td>
</tr>
<tr>
<td></td>
<td>Restrictive physiology</td>
</tr>
</tbody>
</table>

Sohn et al., JACC 1997
Figure 1: Practical approach to assessment and grading of Diastolic Dysfunction

**E/A < 1** without any additional evidence of diastolic dysfunction can be normal about 60 years of age.

**E/A > 1.2** and/or increased LA size without structural heart disease can be seen in young subjects and athletes.

**E/A > 2** combined with one or more parameters from below. Confidence of categorisation increases with increasing number of contributive parameters.

If E/a' is between 9 and 11, additional measurements should be used (see text).