SURVIVAL OUTCOME AND GROWTH OF CHILDREN WITH LANGERHANS CELL HISTIOCYTOSIS: A 20 YEARS EXPERIENCE FROM A SINGLE INSTITUTION IN MALAYSIA

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Name of Candidate: Dr. Ooi Li Lyn [redacted]

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Title of Thesis ("this Work"): Treatment Outcome of Children with Langerhans Cell Histiocytosis: A 20 year's experience from a single Institution in Malaysia.

Field of Study: Paediatric Oncology

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ABSTRACT

Langerhans cell histiocytosis (LCH) is a rare clonal proliferation of immunophenotypically and functionally immature LCH cells which lead to organ damage. The present study describes the data on LCH collected in a single institution in Malaysia over a 20-year period.

Method: This is a retrospective study of patients with LCH being treated in paediatric oncology unit, UMMC. Beginning from 1st January 1997 to 31st December 2015, all new patients with a confirmed diagnosis with biopsy which demostrate CD1a antigenic determinants on the surface of lesional cells, or cells expressed S100 were included. Patient characteristics, presenting clinical features, date of diagnosis, imaging finding, biopsy report, treatment received, outcome, final height when last seen and date of last seen were recorded in a standard data collection sheet.

Results: We analyzed 53 patients who were diagnosed with LCH within the study period. Median age of diagnosis was 24 months (range 0 to 10 years old). Bone was the most frequently affected organ (70%) followed by liver (40%). 20 patients (38%) had single-system involvement (SS), 6 (11%) with multisystem (MS) disease without risk organ involvement (MS-RO$^-$), and 27 (51%) multisystem disease with risk organ involvement (MS-RO$^+$). The 5-year overall survival (OS) rates in the SS, MS-RO$^-$, and MS-RO$^+$ groups were 100%, 100%, and 51.8%, respectively ($P<0.001$). Subjects with MS-RO$^+$ had poorer weight and height at baseline and follow-up than subjects with SS and MS-RO$^-$. Mean height SDS and mean weight SDS were lower in subjects with concomitant DI than those without DI at baseline.

Conclusion: Similar disease and patient characteristic were observed in our children with LCH compared to other centers. Patients in our center are significantly having poor growth which needs urgent attention. Although our results were inferior compared to the major trials, the overall outcome remain optimistic.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank all the patients in this study, without whom this work would not have been materialized. Many thanks to Madam Chan Mo Tsing for her tireless effort in tracing all the chemocards that have been very helpful in terms of my data collection. My heartfelt appreciation goes to Professor Wan Arrifin Abdullah, Dr. Azriyanti Binti Anuar Zaini and Dr. Revathi A/P Rajagopal, my supervisors, who had echoed throughout this whole journey and guided me to the right path. My special gratitude to my family, friends and colleagues who had lend me a helping hand in many ways.

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LIST OF SYMBOLS AND ABBREVIATIONS

CT	Computerised Tomography
EFS	Event Free Survival
HPE	Histopathological Examination
MRI	Magnetic Resonance Imaging
OS	Overall Survival
LCH	Langerhans Cell Histiocytosis
UMMC	University Malaya Medical Centre
MAPK	mitogen-activated protein kinase
ERK	extracellular signal-regulated kinase pathway

CHAPTER 1 : INTRODUCTION AND LITERARTURE REVIEW ON LCH

1.1 Introduction

Histiocytoses is a relatively rare disease which can be classified into either Langerhans cell histiocytosis (LCH) or non-LCHs, depending on the type of cells involved. In LCH, the dendritic cells or macrophages can be characterized by immunohistochemical methods with CD1a and S100 antibodies.[1] The pathogenesis and aetiology remain unclear. The clinical manifestation and course are very variable, from a solitary, self-healing lesion to fatal multiorgan disease involving risk organs, including the liver, spleen, lungs, and the hematopoietic system. The disease can be encountered in any age group but is most often diagnosed in children.

1.2 Genetics

BRAF genes governed cell growth and development .In view of somatic mutation, the *BRAF* protein in affected cell will continuously being active. This overactive protein will cause Langerhans cell to grow and proliferate uncontrollably. Individually, the *BRAFV600E* mutation had been reported in 57–69% of patients with isolated LCH.[1] Recent data support a model in which LCH is driven by pathologic ERK activation ,arising from activating somatic mutation in the mitogen-activated protein kinase (MAPK) pathway. [2]

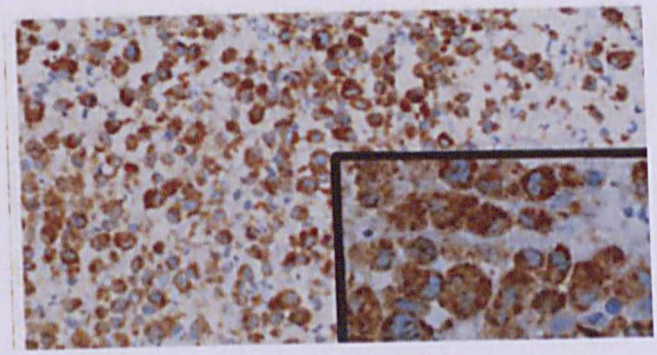

Figure 1.1 BRAF-VE1 immunohistochemical stain demonstrates strong cytoplasmic staining.

1.3 Incidence

The overall incidence rate for LCH was 2.6 cases per million child years. In those under 1 year of age the incidence rate was 9.0 cases per million child years.[3]The descriptive epidemiology of LCH remains poorly documented as there are less than 10 published studies which was conducted on at most about a100 cases which actually reported about incidence rate.[4] The 10 year study in Stockholm County reported the minimal incidence of LCH there was estimated to be 8.9 cases per million children per year.[5]

A nationwide study in Korea reported a total of 603 patients diagnosed with LCH between 1986 and 2010, retrospectively collected from 28 institutions in Korea.[6]

Because of its rarity, single-institution reports always yield a small numbers of recruited patients. For a 5 years study carried out in South India, they reported a total of 40 cases diagnosed with LCH. [7] A group of Brazilian managed to report a total of 37 case diagnosed with LCH in 20 years experience in a single institution.[8]

For our local data, to date, Stomatology Unit, Institute for Medical Research, Kuala Lumpur had reported a total of 17 cases of oral LCH diagnosed between years 1967 till 2007. [9]

All studies showed more male patients diagnosed with LCH but study done in Brazil and United States showed more female patients. However, there is no significant gender preponderance noted in most of the LCH studies.

Most epidemiological studies described LCH in European paediatric populations. There are very few study described LCH among adults. The only study reported by International registry of the Histiocytes Society described 274 adult LCH patients from 13 countries.[10]

The median age of diagnosis was documented as 2-3 years old in most of the literature review. A higher median age of diagnosis was noted in the Korean study as they recruited patients from 0 years old till 23 years of age.

1.4 Risk Stratifications

Based on the classification defined by Histiocyte society, LCH will be differentiates between single system disease (SS-LCH) and multisystem disease (MS-LCH). The classification is mainly based on the extent of organ involvement at diagnosis. In SS-LCH, only one organ or system is involved such as bone (either as a single bone or more than one bone), skin, lymph node (not the draining lymph node of another LCH lesion), hypothalamic-pituitary/central nervous system, or others such as thyroid or thymus. In MS-LCH, two or more organs, or systems are involved either with or without involvement of risk organs. Multisystem with risk organ involvement is described as MS-RO$^+$, which the risk organ include the hematologic system, the spleen, liver and the lungs. Involvement of skull bones, with the exception of the vault is considered " CNS risk" lesion.[11] However, in the upcoming LCH-IV clinical trial, the lung will no longer be considered as a risk organ. [12] For patient who has multisystem involvement but with no risk organ involved, they will be labelled as MS-RO$^-$.

Table 1.1 Risk Stratifications

SS – single system Multifocal bone disease Localized "special site" involvement	Patients with two or more different bones involvement. Patients with "CNS-RISK" lesions with intracranial soft tissue extension or vertebral lesions with intraspinal soft tissue extension
MS- Multisystem MS-RO$^+$ MS-RO$^-$	Multisystem patients with involvement of one or more "RISK" organ Multisystem patients with multiple organs involved but without involvement of "RISK" organs

Table 1.2 Definition of "RISK" organ

Hematopoetic involvement - With or without bone marrow involvement	Anemia: Hemoglobin <10g/dL, infant <9g/dL Leucocytopenia: leucocytes <4 x 10^9/L Thrombocytopenia: platelets < 100x10^9/L
Spleen involvement	Enlargement ≥ 2cm below costal margin (proven by sonography)
Liver involvement	Enlargement > 3cm below costal margin (proven by sonography) and/or liver dysfunction (hyperbilirubinemia, hypoproteinemia, hypoalbuminemia, elevated γGT, alkaline phosphatase, elevated transminases, ascites, edema) and/or histopathological diagnosis
Lung involvement	Typical changes on high resolution computed tomography (HR-CT) and/or histopathological diagnosis

1.5 Clinical Presentation and organ involved

The most common complaint at the time of diagnosis was local pain or swelling followed by skin rash. There are also patient who presented with prolonged fever or incidental finding of organomegaly. The most frequently involved organ will be the skeleton (80%) , followed by the skin and the lymph node. Other organ involvement accounts for a smaller proportion of patients, namely the liver, spleen, hematopoietic system and the pituitary.[6]

Table 1.3 Clinical presentation in different studies

Study	Bone	Hematopoietic system	Liver	Spleen	Lymph node	Skin
Korea[6]	481(80%)	44(7%)	74(12%)	43(7%)	83(14%)	118(20%)
France[4]	191(74%)	13(5%)	11(4%)	11(4%)	NA	86(33%)
England[3]	67(66%)	NA	16(16%)	16(16%)	41(41%)	37(37%)
Sweden[5]	24(83%)	2(7%)	1(3%)	1(3%)	2(7%)	10(34%)
Brazil[8]	25(68%)	NA	9(24%)	7(19%)	17(46%)	17(46%)
India [7]	28(70%)	NA	NA	NA	16(40%)	10(25%)

1.6 Histopathological diagnostic criteria

The diagnosis of LCH is based on histological and immunophenotypic examination of lesional tissue, which mainly involved the identification of the characteristic LCH cells (prominent folds and grooves, fine chromatin, and indistinct nucleoli). However, a definitive diagnosis requires the demonstration of CD1a antigenic determinants on the surface of lesional cells or the finding of Birbeck granules in lesional cells by electron microscopy. If the cells express S100 and at least one of the following: ATPase. Alpha-D-mannosidase, peanut lectin, it is justified to consider LCH as provisional diagnosis. [13] The differential diagnosis for LCH may include Rosai-Dorfman disease, Erdheim-Chester disease and Juvenile xanthogranuloma. However, Rosai-Dorfman disease does not show expression of CD1a. Erdheim-Chester disease shared similar clinical features with LCH, including bone involvement and *BRAFV600E* mutation, but they mainly involved adult onset and histologically characterized by foamy histiocytes without expression of S100 and CD1a. On the other hand , juvenile xanthogranuloma mainly characterized by foamy histiocytes and Touton giant cells.[14]

The most common biopsy site is bone as bone involvement is known to be the commonest site of involvement. In unifocal osseous lesion, histology is essential for confirmation of diagnosis as clinical and radiographic findings are not specific enough. [10] It is also reported that unifocal osseous LCH which was treated with biopsy alone achieved symptom resolution in <4 weeks.[10]

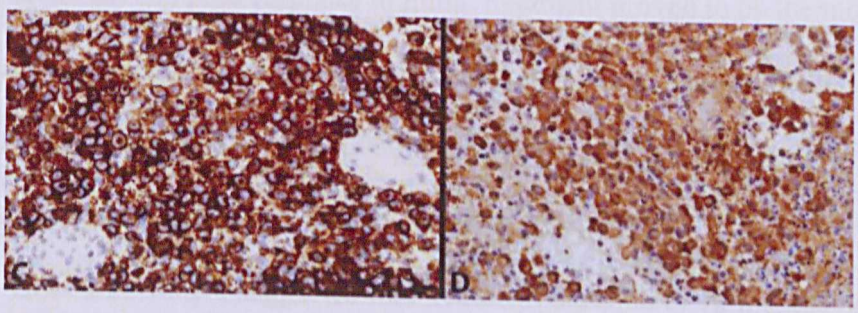

Figure 1.2 The LCH cells are immunoreactive for antibodies directed against CD1a (C) and S100 protein(D).

1.7 Treatment

The Histiocyte Society initiated LCH I- the first international clinical trial for the treatment of multisystem LCH in year 1991. The comparison between monotherapy with Vinblastine and etoposide showed no significant difference with respect to initial response and probability of reactivity and mortality.

There were two multicentre clinical trials which had been run in Austria, Germany, Netherlands and Switzerland between year 1983 and 1990, namely DAX HL-83 and DAL HX-90. These two clinical trials used polychemotherapy protocol included an initial treatment with prednisolone for 6 weeks in combination with vinblastine and etoposide, followed by oral mercaptopurine and 3 weekly pulses of prednisolone, vinblastine, etoposide and methotrexate. The comparison of LCH I and DAL HX-83/90 results showed a clear superiority of combination therapy given for one year with respect to initial response and rate of reactivation. DAL HX-83 trials revealed a low mortality rate but one should keep in mind that this trial had actually included patients with multifocal bone lesion into the "disseminated LCH" group. This group of patients which was known to have excellent prognosis could have skewed the overall results. [15]

LCH II study was started on year 1996 which a new stratification system was adopted, distinguishing between "RISK" patients with involvement of "RISK" organs like liver, spleen, lungs, hematopoietic system or age under 2 years old. Risk organ involvement and poor response to initial treatment proved to be the most crucial prognostic factor. Etoposide had not shown to add any therapeutic benefit with respect to response, survival and reactivation frequency. It was then withdrawn from the protocol considering its potential leukemogenicity. [11]

LCH-III protocol suggested that treatment duration of 12 months reduces the rate of reactivation as compared to 6 months treatment duration. It is partly due to patient with

MS-LCH may have a very variable clinical course. Patient who presented with multi-systems disease (regardless of risk organ involvement) will be started on standard initial therapy, which consists of a combination of prednisolone and vinblastine. They will be a reassessment at the end of the initial 6 week course of therapy. Patient with risk organ involvement at diagnosis who showed improvement and patient without risk organ involvement at diagnosis, who showed no improvement, are recommended to get a second course of treatment with prednisolone and vinblastine. [11]

Patients who have complete disease resolution after 6-12 weeks of initial therapy will continue with maintenance therapy. Maintenance therapy consists of pulses of vinblastine and prednisone every 3 weeks and daily 6-mercaptopurine (6MP) for a total treatment duration of 12 months.[11]

Patients with multifocal bone, special site and CNS-risk lesion belongs to other subgroups. Systemic treatment carried the risk to cause permanent consequences and disabilities, but clinically proven to reduce the reactivation rate. They are recommended to receive the similar treatment as per multisystem group but without the 6MP as the maintenance drugs.

Table 1.4 Comparing treatment using different protocols

Treatment trials	Treatment protocol	Outcome
LCH I study (year 1991-1995)	24 weeks vinblastine/etoposide and a single initial dose of corticosteroid.	- Etoposide increased risk of secondary malignancy. - Response rate at 6 weeks was low – 51%
LCH II study (year 1996-2001)	Patients were divided into low and high risk group. The high risk patients were further randomized to receive Arm A or Arm B treatment. Arm A- prednisolone + vinblastine. Arm B- prednisolone + vinblastine + etoposide	- the addition of etoposide does not improve survival. - patients with risk organ involvement have significant disease-related mortality - the disease reactivation rate in the low-risk group is still high.
LCH III study (year 2001-2008)	Etoposide was withdrewn because of its leukemogenic potential. Intensification of initial therapy for patients who did not achieve resolution after 6 weeks of therapy by delivering a second 6-week course of initial therapy. For low-risk group trial, prolongation of treatment duration from 6 to 12 months .	- preliminary unpublished evaluation suggest that the overall survival is higher. - Prolongation of therapy in low risk group may improve the probability of disease reactivation- i. free survival.
JLSG-96 trial (Japan Langerhans Cell Histiocytosis Study Group-96)	Three cycles of prednisolone, vincristine, and cytarabine given over 6 weeks, which was followed by continuation (add low dose methotrexate)therapy, giving a total treatment duration of 7.5 months. Patients who did not respond or progressed were treated with a more intensive salvage regimen consisting of prednisolone, vincristine, doxorubicin, and cyclophosphamide.	- Excellent5-year OS -94.4% . - High reactivation rate due to short duration of therapy.
French LCH Study Group	A combination of cladribine and cytarabine was used in patients with MS-LCH who had severe disease (involvement of risk organs) refractory to standard therapy.	70% cure in this cohort of patients with the most severe disease.

1.8 Follow up

Children with LCH, who had completed treatment, will require long term follow up which focus on ongoing surveillance for recurrence and treatment-related complications. LCH-III protocol had implemented a schedule for follow up investigation after the end of therapy. During the first year of disease, patient should be

following up every 6 weekly. Clinical examination should be carried out during each follow up. Height, weight and pubertal status also need to be assessed every 6 monthly.[13]

1.8.1 Blood investigations surveillance

Blood investigation should be taken every 3 monthly for patients who have had respective organ involvement, for example blood count, ESR, liver and renal function test, urine and serum osmolality. [13] The median age of developed DI was reported as 3.9 years after onset of LCH. [16]

1.8.2 Liver and pulmonary surveillance

For patient with liver involvement, ultrasound liver need to be repeated every 6 monthly for the first year of diagnosis and subsequently annually. As for patient with lung involvement, it is recommended to do lung function test and HRCT thorax every 6 monthly for the first year of diagnosis. [13]

1.8.3 Radiographical surveillance

Patients with diabetes insipidus and other endocrinopathy should have their MRI brain done annually. Radiographs of the bone lesion should be done if suspected any new lesions or reactivation. [13]

1.9 Survival outcome

For the first single institutional Korean study done in year 1986 till 2007, they reported the overall survival (OS) of the total study population as 97.1%. Patient with single system involvement showed 100% survival rate. [17]A subsequent Korean nationwide study which involved 28 institutions reported the 5 year overall survival rates in the entire cohort as 95.4%. It was further analyzed based on the risk stratification group, SS, MS-RO$^+$ and MS-RO$^-$, which showed 5 year OS rates of 99.8%, 77% and 98.4% respectively. [6]For patient with bone involvement, the 5 year OS rate was significantly higher (96.3%) compared to those with extraosseous disease site (80.7%). For patient with pulmonary involvement only, the 5 year OS rates dropped till 83%. [18]

The overall survival rate was recorded as 79% at 1 year, 74% at 3 years and 71% at 5 years in a 45 years nationwide study carried out in Northwest England. There were no deaths beyond 5 years among the cohort. They noted that survival had improved over time, from a 5-year OS of 57% for the period 1954-168 till 74% for cases diagnosed in year 1985-1998. They reported a poor 5-year OS rate for those with liver or spleen involvement, only 25%.[3]

France study between year 2001-2004 showed a 1 year and 2 year OS rate as 99% , partly due to the short study period. [4]

As for the 20 year Brazilian study, OS for the whole group was 88.5%. OS was significantly higher for patients with single-system (100%) when compared to those with multisystem disease (77.2%). [8]

A 10 year study done in United States which collected data from 18 population-based cancer registries, reported the 5-year Relative Survival (RS) as 90%. There is no significant difference were observed in survival rates according to race, ethnicity, or socioeconomic variables.[19]

Table 1.5 : Survival outcome of LCH subjects

Study	Overall survival
Argentina[18]	59% (only confined to multisystem group)
France [4]	99% (1 and 2 year OS)
England [3]	71% (5 year OS)
Brazil[8]	88.5% (5 year OS)
Korea[6]	95.4% (5 year OS)
United States[19]	90% (5 year OS)

1.9.1 Event free survival

In a Brazilian study, they reported the event free survival at 10 years for their patient to be 32.5%. When considering the risk stratification group, the EFS at 10 years for single system group is reported to be 47.1% and 14.1% for the multisystem group.

1.10 Disease outcome and complications

LCH can results in sequelae which involve various tissue sites. Some of them will present at diagnosis whilst other may only manifest after several years. Hence, it is crucial to monitor these patients until adult life. [12]

1.10.1 Endocrine complications

In a retrospective nationwide multi-center study done by French LCH group, they noted 25% of their patient developed endocrine dysfunction. Diabetes insipidus is the most frequent endocrine complications seen in LCH patient, reported as 24%, followed by growth hormone deficiency (10%) [16]. The cumulative risk of developing DI was 26.0% after 14 years from diagnosis of LCH. [20]The postulated pathogenesis of DI involved infiltration and/or scarring of the hypothalamus-pituitary-axis or autoimmune process against the vasopressin.[21]Any child whose growth is below expection should be investigated extensively. The cumulative risk of being diagnosed with growth retardation was reported as 17.6% after 14 years from diagnosis.[20] Others include delayed puberty and panhypopituitarism. Patients with endocrinopathy, during the first 3 years after chemotherapy, were also noted to have more recurrences. [16]

Table 1.6 Others complication

Orthopedic complications	- rely on the affected sites. Vertebral collapse and facial asymmetry were the most frequently reported.[20] Surprisingly, study reported that radiotherapy did not appear to be a significant risk factor for orthopaedic sequelae.
Neurological complications	- at risk in developing neuropsychological sequelae, in particular cerebellar ataxia and learning difficulties.
Respiratory complications	-Lung fibrosis was reported in 33% of those who had known lung involvement LCH. [20]
Hepatological complications	-at risk to develop sclerosing cholangitis which can progress to liver cirrhosis later in life.

CHAPTER 2 : RATIONALE AND OBJECTIVES

2.1 Rationale

LCH is a rare proliferative disorder of pathological Langerhans cell, for which the diagnosis remained challenging as presenting symptoms are varied one to another. LCH may present from an isolated skin rash or a single bony lesion to catastrophic multi-organ failure. Many milder case were actually went un-diagnosed or delayed in diagnosis. University Malay Medical Centre (UMMC) is the first hospital in Malaysia to have a paediatric oncology unit and a pioneer in treating children with malignancy. As such, UMMC not only cater for the need of our local population, it also receives referral from all over the country as well as overseas. In addition to that, there is no audit or studies done for the past for this disease.

2.2 Objectives

2.2.1 Primary objective :

1. To analyse the survival outcome of children with Langerhans Cells Histiocytosis in UMMC during the study period (1st January 1997 until 31st December 2016)

2.2.2 Secondary objective :

1.To review the growth and endocrinal disturbances associated with Pediatric Langerhans Cell Histiocytosis (LCH)

CHAPTER 3: METHODOLOGY

3.1 Study design

This is a retrospective descriptive, cross sectional, cohort study of patients admitted and/or being treated in paediatric oncology unit, UMMC for LCH.

3.2 Patient selection

3.2.1 Inclusion criteria

The study period was from 1st January 1997 till 31st December 2016. All new patients with a confirmed diagnosis of LCH by biopsy, who presented to UMMC during the stipulated period of time above.

3.2.2 Exclusion criteria

For the secondary objectives, all patient records and case notes will be reviewed and analysed.

For the primary objective, patient with the following criteria is excluded from the analysis :

i) Patient who does not have a confirmed biopsy diagnosis

ii) Patient who refused treatment after diagnosis

3.3 Method

Children who were diagnosed with LCH were first identified in the paediatric oncology registry database. Case notes were traced from the medical record department while chemocard were identified in paediatric oncology unit and reviewed. Data collection was done using a standard data collection sheet. Details of patient, demographic profiles, signs and symptoms at presentation, diagnosis, risk stratification group, growth at diagnosis, growth when last seen and treatment outcome were obtained and recorded.

3.4 Ethical Approval

This study was approved by UMMC research ethic committee. (MECID ID no 20166-2527)

3.5 Data Analysis

Data were analysed using Statistical Package for Social Science (SPSS) version 20.0. Baseline patient demographic value, height, weight at diagnosis and height and weight during follow up were presented in mean with standard deviation as it was normally distributed. Whereas age of diagnosis and follow up time were described with median and range since it was not normally distributed. Categorical data such as gender, ethnicity, presenting signs and symptoms, were expressed as frequency with percentages in parentheses. Categorical data were compared with chi-square test or exact alternatives where applicable. To study the outcome of children with LCH, survival analysis using Kaplan-Meier method were applied, differences were tested by means of the log-rank test, a $p<0.05$ are considered as significant.

3.5.1 Event Free Survival (EFS)

Defined as the time from diagnosis to a relapse or detection of disease progress or death (whichever occur first). Patient without events were censored at their last date of follow up.

3.5.2 Overall survival

Only death of any cause was counted as an event. Patients who were still alive or lost of follow up were censored at their last date of follow up.

3.5.3 Survival time

Defined as the time from initial diagnosis to death of any cause.

3.5.4 Follow up time

Defined as the time from initial diagnosis to the last date of follow up.

3.6 Flowchart for data collection

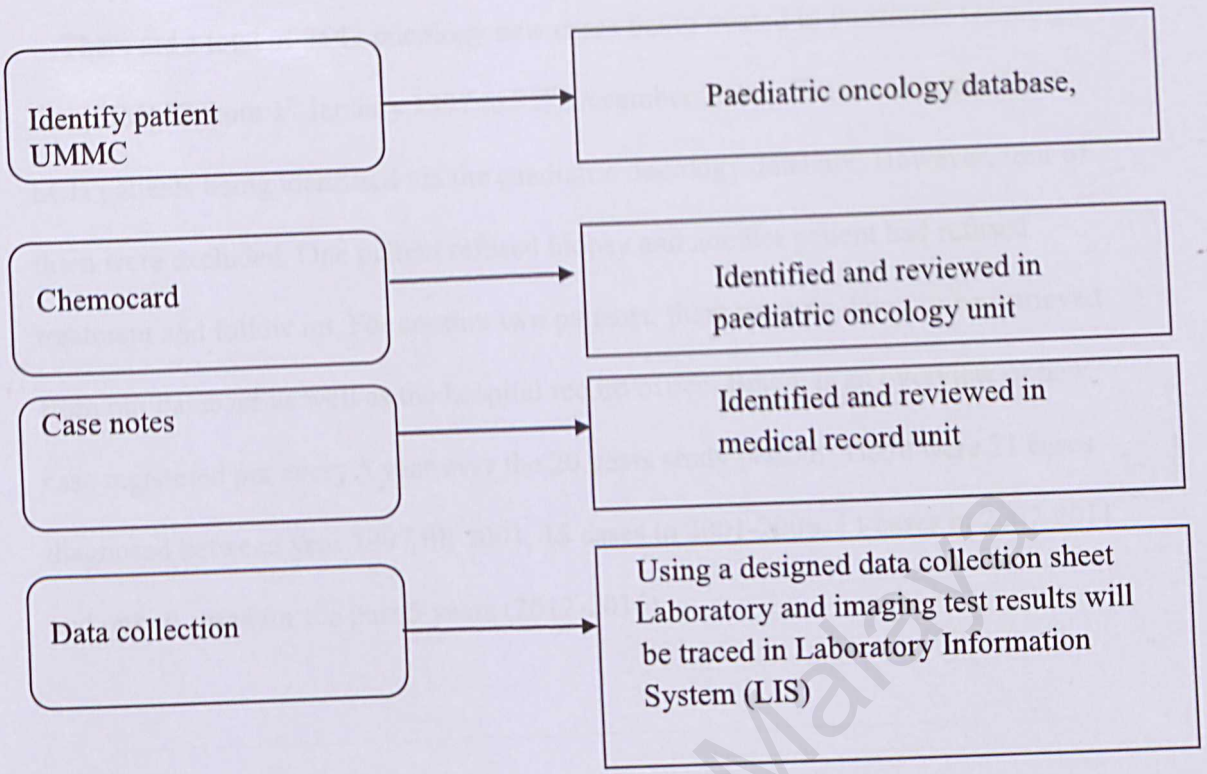

Figure 3.1: Flowchart for data collection for LCH patients in UMMC

3.7 Cohort of patients

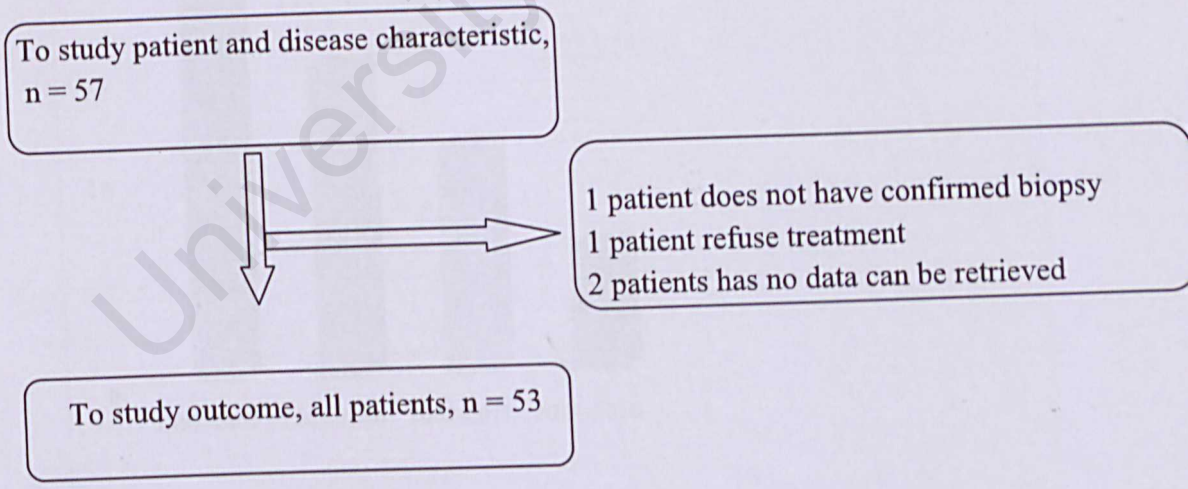

Figure 3.2: cohort of LCH patients in UMMC

CHAPTER 4: RESULTS

There are a total of 2542 oncology new cases being treated in Paediatric Oncology Unit, UMMC from 1st January 1997 to 31st December 2016. There were only 57 of LCH patients being identified via the paediatric oncology database. However, four of them were excluded. One patient refused biopsy and another patient had refused treatment and follow up. For another two patients, there were no data can be retrieved from our database as well as the hospital record office. Below is an overview of new case registered per every 5 year over the 20 years study period. There were 21 cases diagnosed between year 1997 till 2001, 15 cases in 2001-2006, 11 cases in 2007-2011 and only 6 cases for the past 5 years (2012-2016).

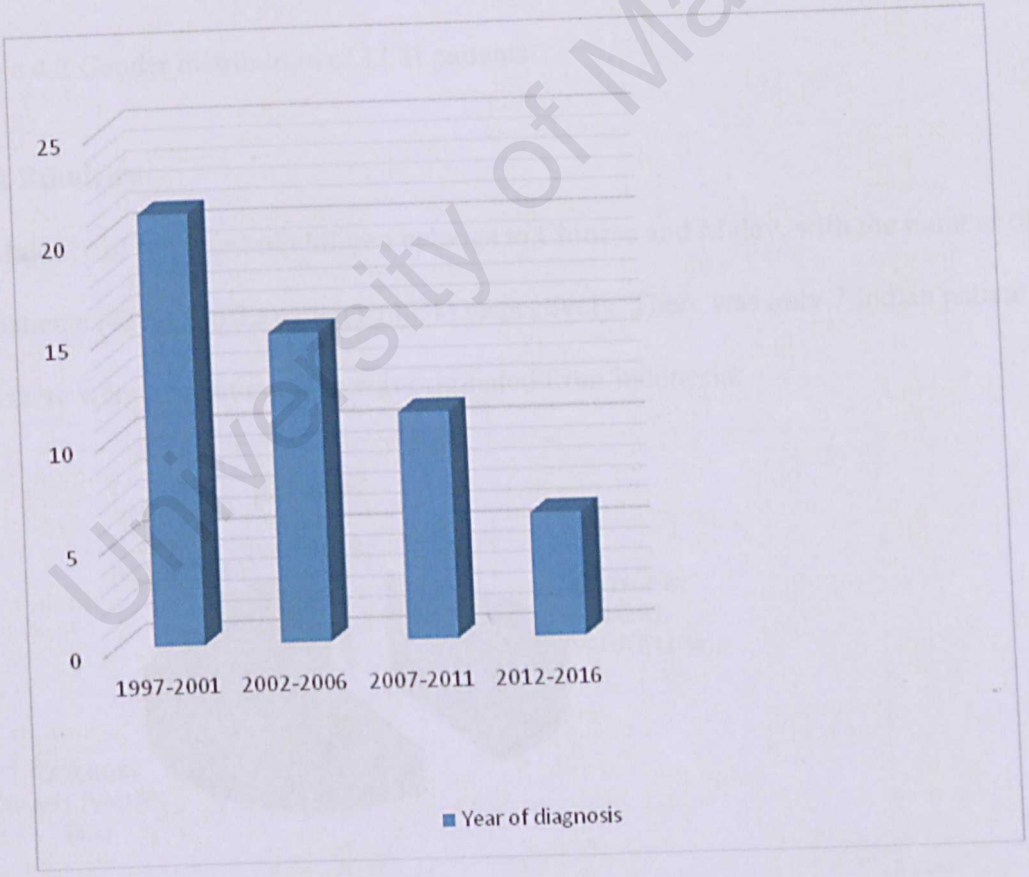

Figure 4.1: New case of LCH registered every 5 year interval in UMMC, n=53

4.1 Demographic Features

4.1.1 Gender

Among the total of 53 patients, 34 of them were male (64 %) and 19 of them were female (36%).

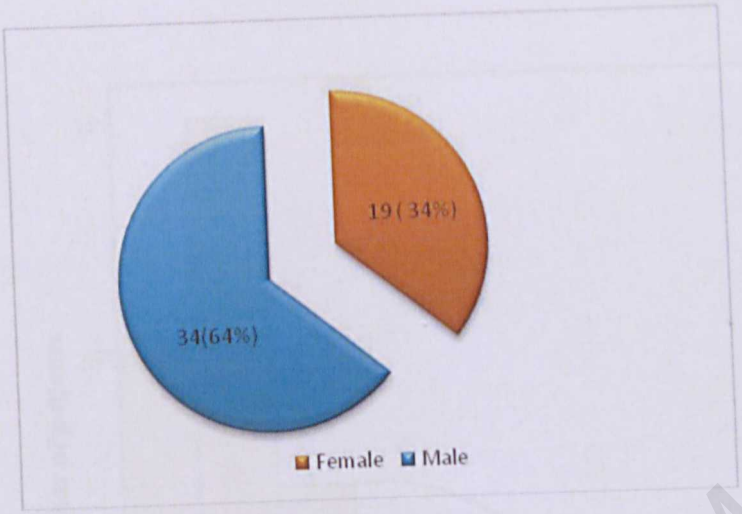

Figure 4.2 Gender distribution of LCH patients

4.1.2 Ethnicity

Majority of the affected children belongs to Chinese and Malay, with the number of 23 patients (43%) and 22 patients (42%) respectively. There was only 7 Indian patients and there were one patient who was originated from Indonesia.

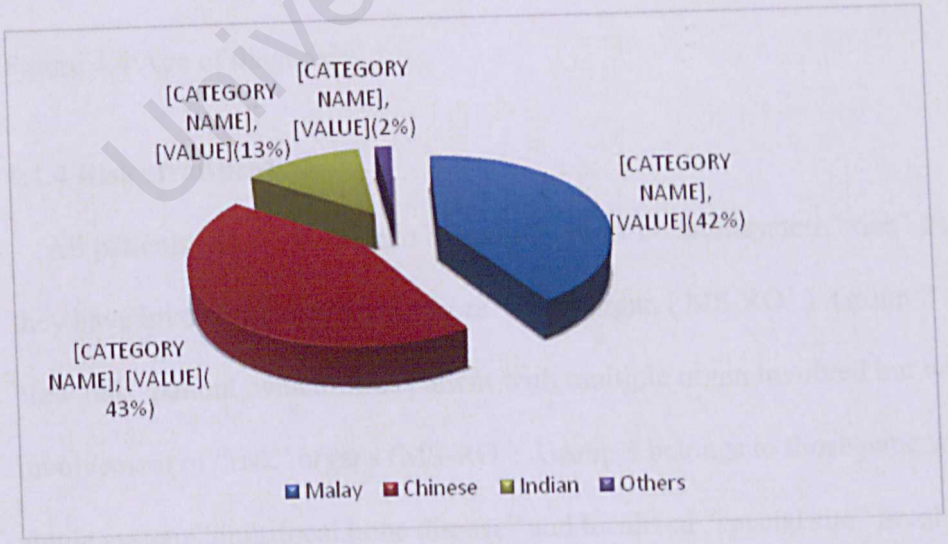

Figure 4.3 Ethnicity distribution of LCH patients

4.1.3 Age of diagnosis

The median age of diagnosis is 2.0 years old (IQR 3.9 years old). The median age was used to represent the data as it is not normally distributed. The youngest patient was diagnosed at the age of one month old while the oldest patient was 10 years old.

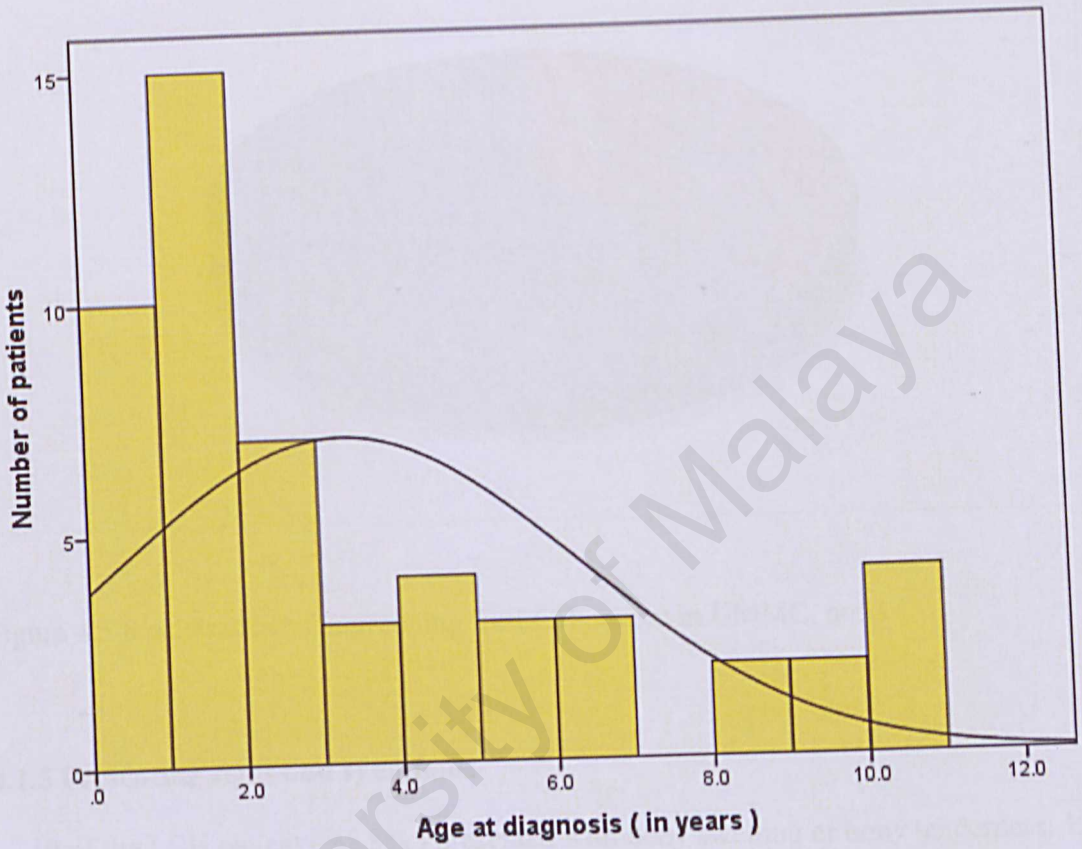

Figure 4.4 Age of diagnosis

4.1.4 Risk stratification

All patients were divided into 3 group – group 1- multisystem "risk" patient, which they have involvement of one or more " risk" organ (MS-RO^{+}). Group 2 – multisystem "low risk" patient, which these patient with multiple organ involved but without involvement of "risk" organs (MS-RO^{-}). Group 3 belongs to those patient who has single system "multifocal bone disease" and localized "special site" involvement (SS). Majority of the patients belong to multisystem "risk" group (MS-RO^{+}), 27 out of 53 patients (50.9%), followed by unisystem multifocal bone disease or special site group

(SS) which constitute of 20 patients (37.7 %). Only 6 patients belong to multisystem low risk group (MS-RO^{-}).

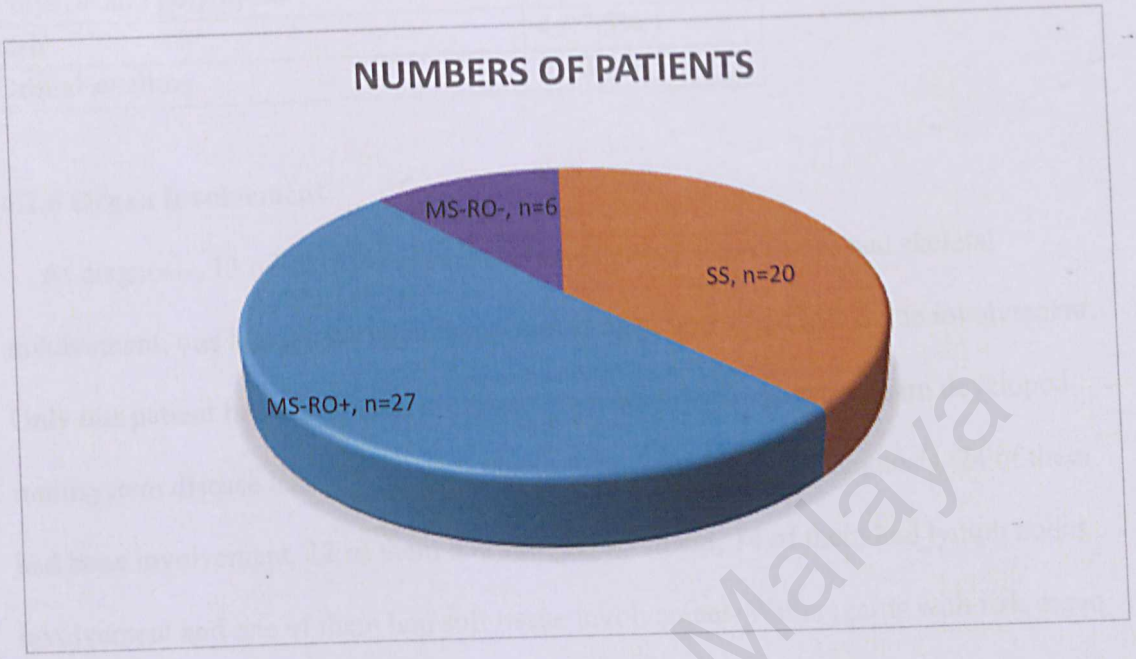

Figure 4.5 Risk stratification grouping for LCH patient in UMMC, n=53

4.1.5 Presenting signs and symptoms

19 of the LCH patient (35.8%) presented with bony swelling or bony tenderness. 12 (22.6%)of them had reported to have scalp or skin nodules which turn up to be lymph nodes swelling . There is another group of patient who presented as prolong fever as well, which contribute to 11 out of 53 patient (20.8%). There were 5 patients (9.4 %) who presented as polyuria and polydipsia and was diagnosed to have diabetes insipidus after significant water deprivation test. Only four patients who presented with skin rash. Of note, there were 2 patients who actually presented with orbital swelling and the biopsy turn out to be LCH.

Table 4.1 Presenting signs and symptoms of LCH patients in UMMC, n=53 (%)

Bony swelling or bony tenderness	19 (35.8%)
Scalp or skin nodules, lymphadenopathy	12 (22.6%)
Prolong fever	11 (20.8%)
Polyuria and polydipsia	5 (9.4%)
rash	4 (7.5%)
Orbital swelling	2 (3.8%)

4.1.6 Organ involvement

At diagnosis, 13 of 20 (65%) patients with single system disease had skeletal involvement, one had lymph node involvement only, and 5 had soft tissue involvement. Only one patient had sorely skin involvement in SS disease. None of them developed multisystem disease later. 33 patients had multisystem disease at diagnosis. 24 of them had bone involvement, 12 of them had skin involvement, 14 of them had lymph nodes involvement and one of them had soft tissue involvement. With regards with risk organ involvement, 21 of them had liver involvement, 10 with spleen involvement and 11 had marrow involvement. Lung as initial risk organ involvement was seen in eight patients.

Table 4.2 Organ system involvement of LCH at diagnosis

Organ system	Number of patients at diagnosis
Bone , unifocal and multifocal	37 (70%)
Skin	12 (23%)
Liver	21 (40%)
Spleen	10(19%)
Bone marrow	11(21%)
Lymph nodes	14(26%)
Lungs	8(15%)
Soft tissues	6(11%)

4.1.7 Biopsy results

Based on the histological report by biopsy, the diagnosis of LCH was defined as definitive in 22 patients of 53 (42%) by demonstrating CD1a positive cells in biopsies from bone, lymph node and liver. The remaining 31 patients (58%) had a presumptive diagnosis of LCH as their biopsies had characteristic morphology and phenotype recognized and the cells express S100.

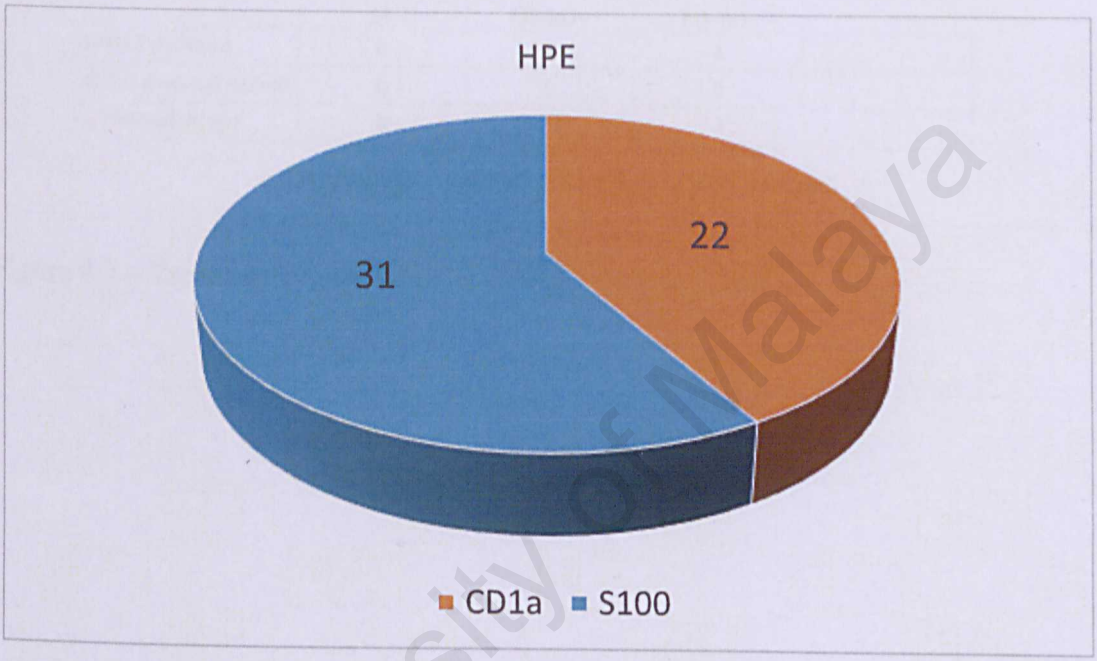

Figure 4.6 – biopsy results of LCH patient in UMMC, n=53

4.1.8 Treatment

Among patient with multisystem risk organ involvement, all of them received chemotherapy – either LCH II or LCH III porotocol. However, in those multisystem low risk patient, 4 of them received chemotherapy and two of them was managed conservatively. Six patients in single system group received chemotherapy, another six of them actually received intralesional steroid , and eight of them received no treatment.

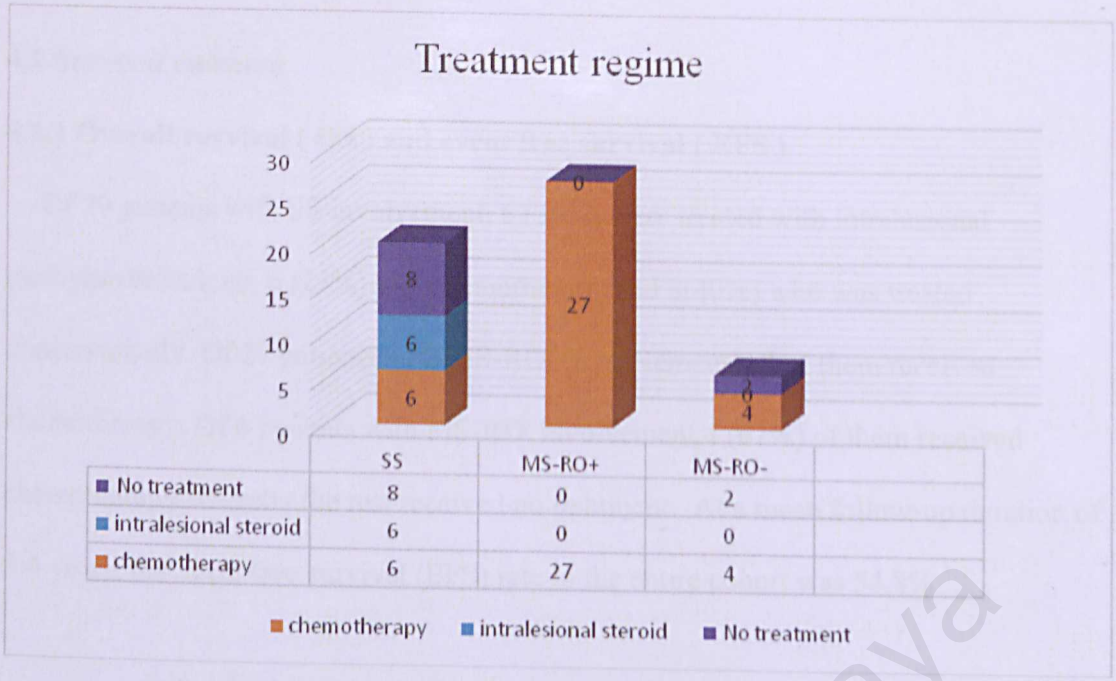

Figure 4.7 – Treatment regime of LCH patient in UMMC, n=53

4.2 Survival outcome

4.2.1 Overall survival (OS) and event free survival (EFS)

Of 20 patients with SS involvement, 6 (35%) were treated with intralesional methylprednisolone, 6 (25%)with chemotherapy and 8(40%) who was treated conservatively. Of 27 patients with MS-RO^{+} involvement, all of them received chemotherapy. Of 6 patients with MS-RO^{-} involvement,4 (67%) of them received chemotherapy whereby the rest received no treatment. At a mean follow-up duration of 5.4 years, the event free survival (EFS) rate in the entire cohort was 54.8%

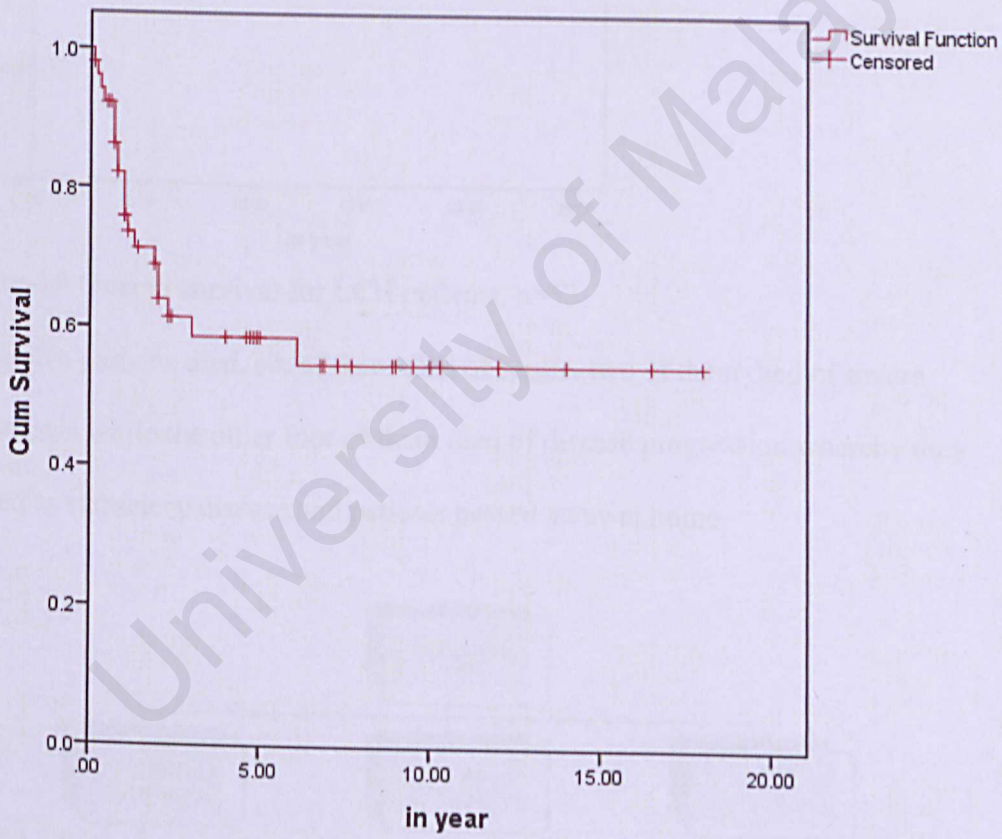

Figure 4.8 Event free survival for LCH patients, n=53

The 5-year overall survival (OS) rates in the entire patient cohort was 74.7%. The 5-year OS rates of the SS, MS-RO$^-$, and MS-RO$^+$ groups were 100%, 100 %, and 51.8%, respectively (P<0.001).

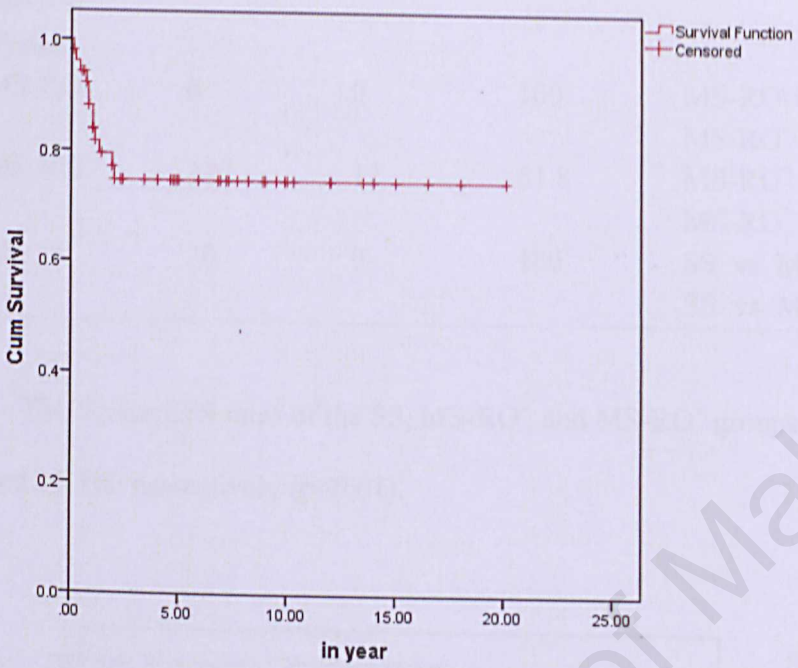

Figure 4.9 Overall survival for LCH patients, n=53

Twelve patients died, six of them died of sepsis, two of them died of severe pneumonia while the other four of them died of disease progression whereby they treated as refractory disease and patients passed away at home.

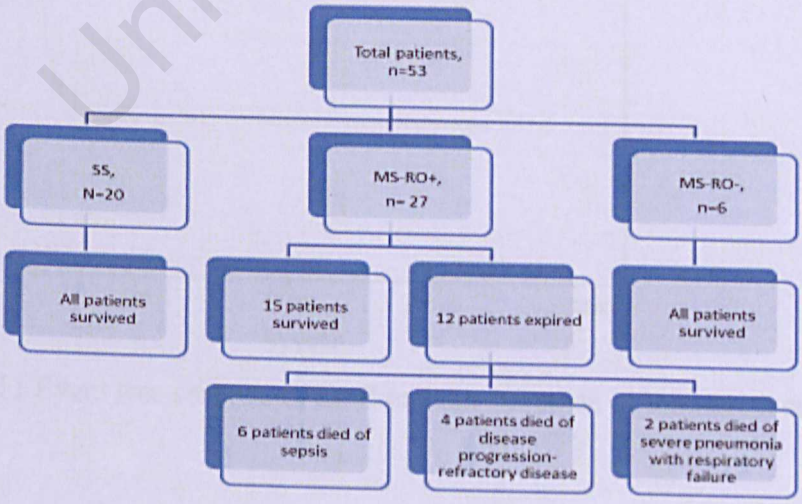

Figure 4.10 Flow chart showing the survival status of LCH patients

Table 4.3 Survival rates and comparison of survival experience

Variable	Number of patient *n*	Number of Events *n*	5 years survival %	Comparison	*p*-value[a]
Overall survival System	53	12	74.7		
MS-RO$^-$	6	0	100	MS-RO$^-$ vs MS-RO$^+$	0.05
				MS-RO$^-$ vs SS	<0.01
MS-RO$^+$	27	12	51.8	MS-RO$^+$ vs MS-RO$^-$	0.05
				MS-RO$^+$ vs SS	0.001
SS	20	0	100	SS vs MS-RO$^-$	<0.01
				SS vs MS-RO$^+$	0.001

The 5-year EFS rates of the SS, MS-RO$^-$, and MS-RO$^+$ groups were 80.7%, 100%, and 25.3%, respectively ($p<0.01$).

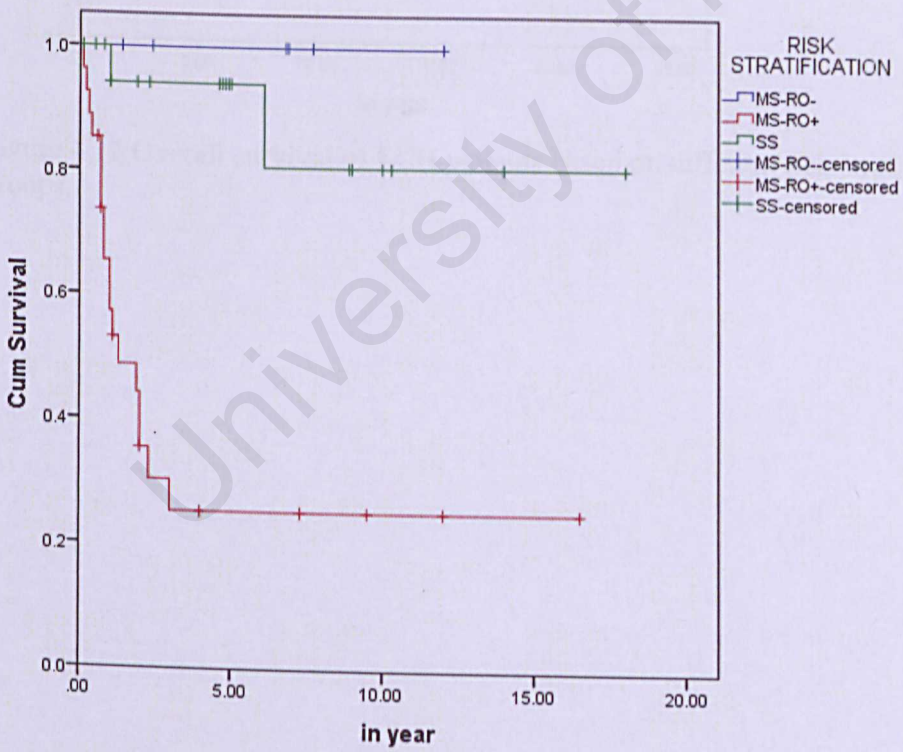

Figure 4.11 Event free survival of LCH patients based on different risk stratification groups.

The 5-year OS rates of the SS, MS-RO$^-$, and MS-RO$^+$ groups were 100%, 100%, and 51.8%, respectively ($p<0.05$).

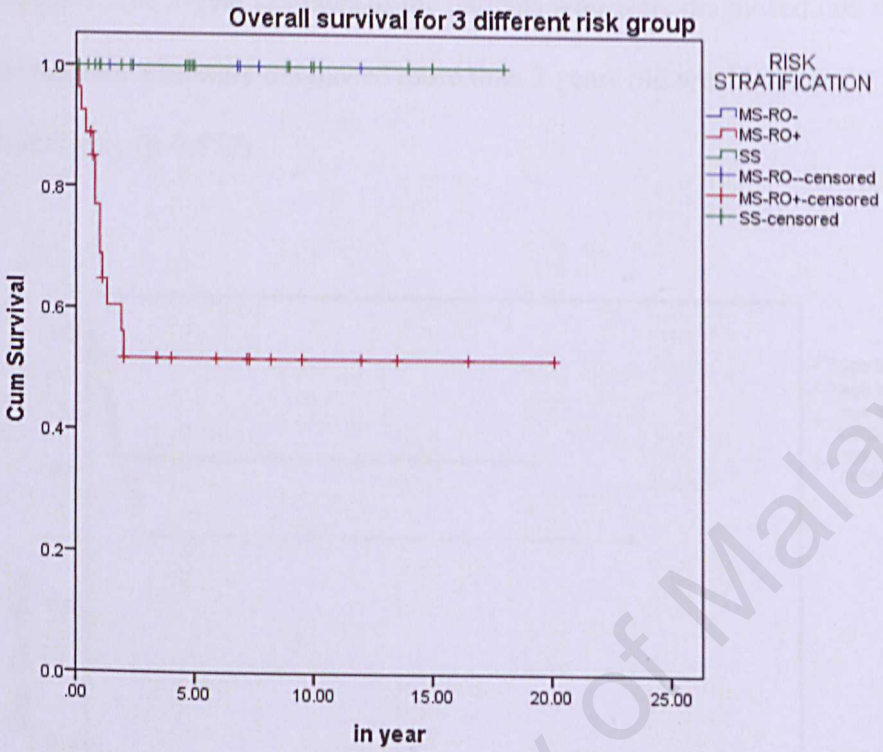

Figure 4.12 Overall survival of LCH patients based on different risk stratification groups.

Further analysis was done to compare survival rate for LCH patients based on the age of diagnosis. There were 30 patients who was diagnosed with LCH at the age of less than 2 years old and the rest of them (23) were more than 2 years old at the age of diagnosis. The 5-year OS rates of the patients who were diagnosed less than 2 years old and patients who were diagnosed more than 2 years old are 70.4% and 81.6%, respectively (p-0.577).

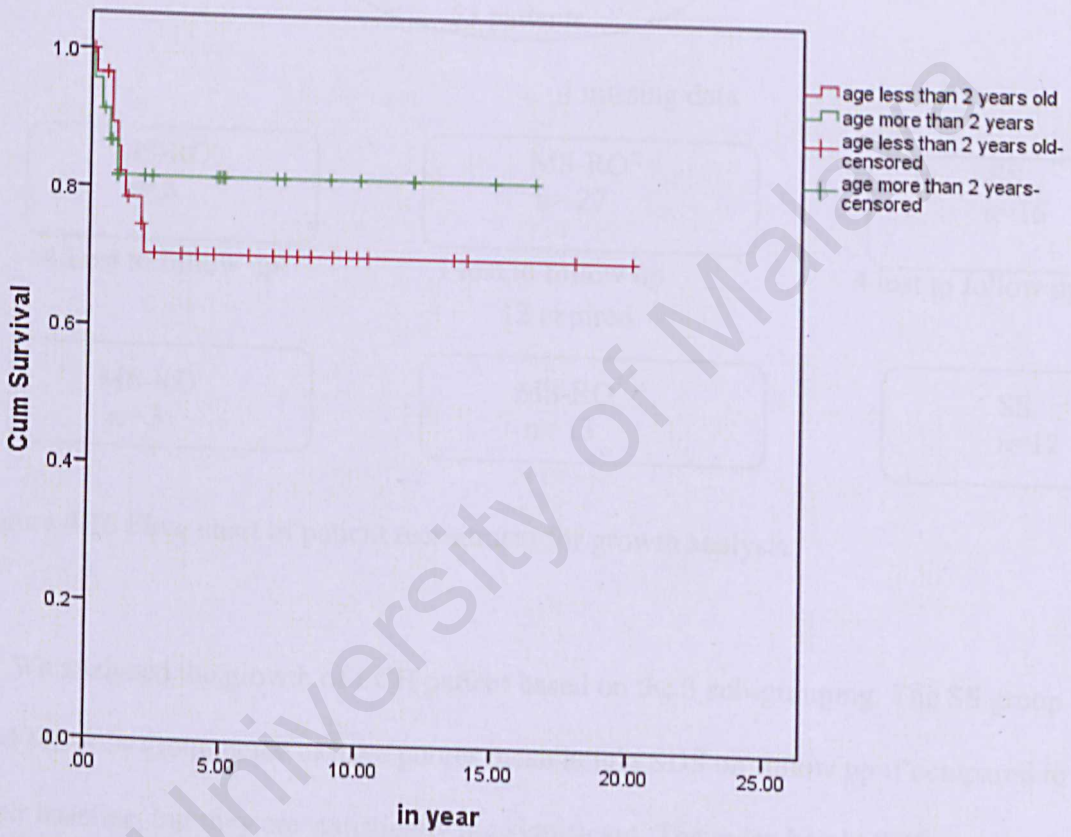

Figure 4.13 The Kaplan-Meier analysis of overall survival for patients by age of diagnosis.

4.3 Growth of LCH patient

The growth of the LCH patient was reported as mean and SD as it was normally distributed. Serial follow-up records were available for only 28 patients, 4 missing data, remaining 9 were lost to follow up and 12 had expired. The 12 patients who expired belonged to the multisystem group with risk organ involvement.

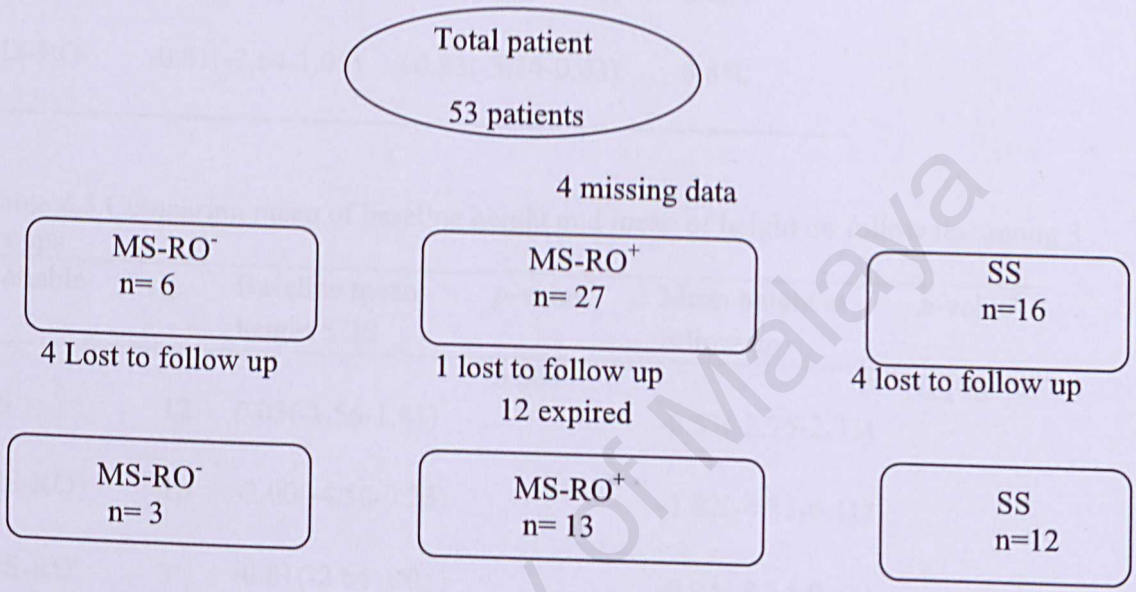

Figure 4.16 Flow chart of patient recruitment for growth analysis

We analyzed the growth of LCH patient based on the 3 sub-grouping. The SS group and MS-RO- group noted to have poorer mean height SDS on follow up if compared to their baseline, but they are statistically not significant. The mean height SDS was noticed to be much more lower in group of multisystem with risk organ involvement ($MS\text{-}RO^{+}$), at baseline and during follow up, if compared to the other 2 group. Using One-way Anova, difference of baseline mean height SDS between the 3 risk stratification groups is statistically significant, p-value – 0.004.However, difference of mean height SDS on follow up between the 3 risk stratification groups is statistically not significant, p-value -0.178

Table 4.4 Comparing mean of baseline height SDS with height SDS at follow up for 3 different groups

Grouping	Baseline mean height SDS	Mean height SDS on follow up	p-value
SS	0.03(-1.56-1.81)	-0.57(-2.75-2.73)	0.288
MS-RO+	-2.00(-4.56-0.33)	-1.82(-4.33-0.11)	0.625
MS-RO-	-0.81(-2.64-1.05)	-0.93(-3.34-0.43)	0.850

Table 4.5 Comparing mean of baseline height and mean of height on follow up among 3 groups

Variable	*n*	Baseline mean height SDS	*p*-value[a]	Mean height at follow up	*p*-value[a]
SS	12	0.03(-1.56-1.81)	0.004	-0.57(-2.75-2.73)	0.178
MS-RO+	13	-2.00(-4.56-0.33)		-1.82(-4.33-0.11)	
MS-RO$^-$	3	-0.81(-2.64-1.05)		-0.93(-3.34-0.43)	

Note: [a]One-way ANOVA test
n= Frequency, *df*=Degrees of Freedom

The mean weight SDS on follow up are noticed to be lower in SS group and MS-RO- group when compared to baseline, though they are statistically not significant. The mean baseline weight SDS was noticed to be much more lower in group of multisystem with risk organ involvement (MS-RO$^+$), if compared to the other 2 group. Using One-way Anova, difference of baseline mean weight SDS between the 3 risk stratification groups is statistically significant, p-value – 0.015. However, difference of mean weight SDS on follow up between the 3 risk stratification group is statistically not significant, p-value -0.571.

Table 4.6 Comparing mean of baseline weight SDS with weight SDS at follow up for 3 different groups

Grouping	Baseline mean weight SDS	Mean weight SDS on follow up	p-value
SS	0.08(-2.03-1.89)	-0.62(-4.90-1.61)	0.193
MS-RO+	-1.81(-4.01-0.32)	-1.23(-4.04-0.67)	0.213
MS-RO-	-1.60(-4.73-1.62)	-1.64(-4.84-1.79)	0.744

Table 4.7 Comparing mean of baseline weight and mean of weight on follow up among 3 groups

Variable	*n*	Baseline mean weight SDS	*p*-value[a]	Mean weight at follow up	*p*-value[a]
			0.015		0.571
SS	12	0.08(-2.03-1.89)		-0.62(-4.90-1.61)	
MS-RO+	13	-1.81(-4.01-0.32)		-1.23(-4.04-0.67)	
MS-RO-	3	-1.60(-4.73-1.62)		-1.64(-4.84-1.79)	

Note: [a]One-way ANOVA test
n= Frequency, *df*=Degrees of Freedom

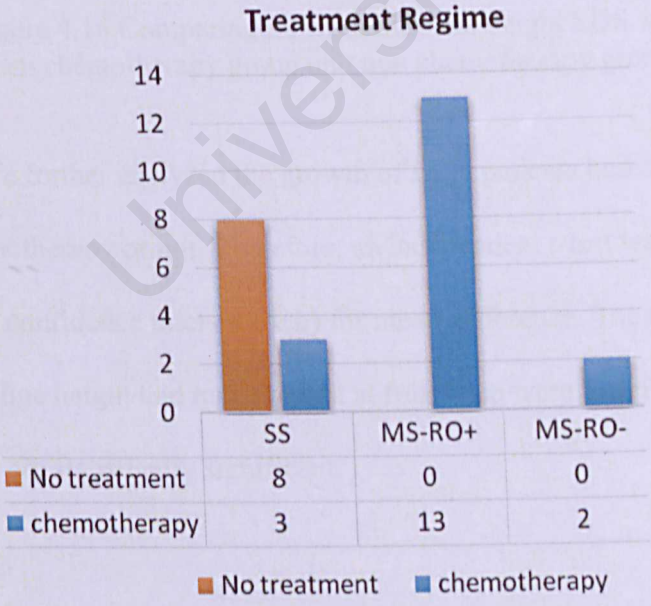

Figure 4.15 Treatment regime for 3 stratification group for growth analysis

We further analyzed the growth of LCH patients based on treatment regime whether they received chemotherapy or not.

Table 4.6 Comparing mean of baseline height SDS with height SDS at follow up between chemotherapy group and non chemotherapy group

Variable	*n*	Baseline mean height SDS	*p*-value[a]	Mean height at follow up	*p*-value[a]
			0.002		0.012
Chemotherapy group	19	-1.64(-4.56-1.05)		-1.82(-4.33-0.43)	
Non chemotherapy group	9	0.33(-0.85-1.81)		0.15(-2.29-2.73)	

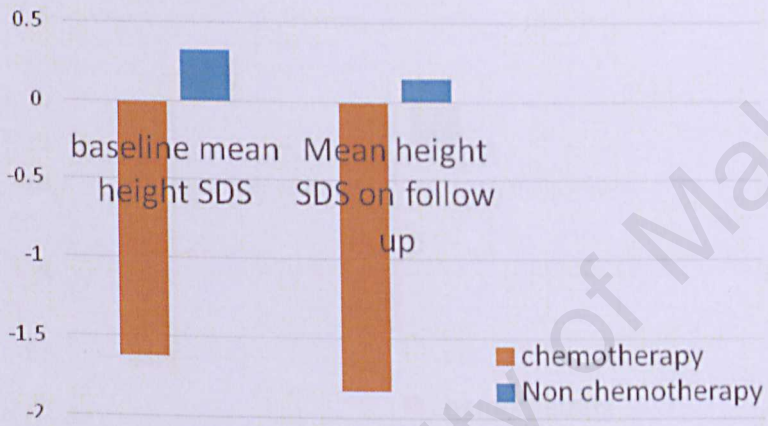

Figure 4.16 Comparing mean of baseline height SDS with height SDS at follow up between chemotherapy group and non chemotherapy group

We further analyzed the growth of LCH patients based on whether they received chemotherapy or not. Therefore, an independent *t*-test was run on the data as well as 95% confidence interval (CI) for mean difference. The results showed that mean baseline height and mean height at follow up were lower in the chemotherapy group and they are statistically significant.

Table 4.7 Comparing mean of baseline weight SDS with weight SDS at follow up between chemotherapy group and non chemotherapy group

Variable	*n*	Baseline mean weight SDS	*p*-value[a]	Mean weight at follow up	*p*-value[a]
			0.006		0.162
Chemotherapy group	19	-1.57(-4.73-1.70)		-1.34(-4.90-1.79)	
Non chemotherapy group	9	0.28(-1.69-1.89)		-0.33(-2.55-1.61)	

Note:[a]Independent *t* test

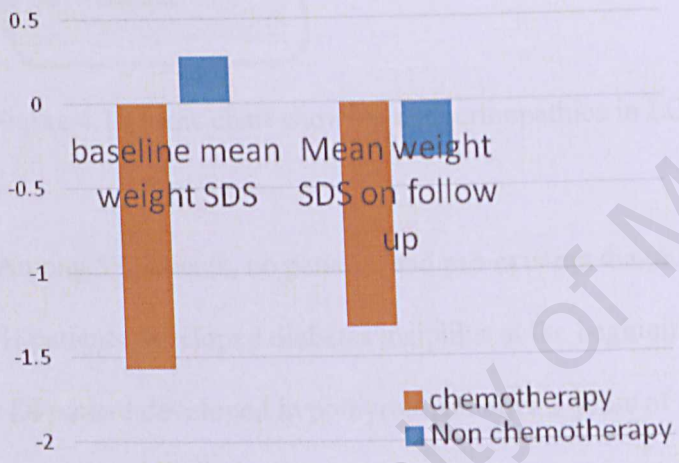

Figure 4.17 Comparing mean of baseline weight SDS with weight SDS at follow up between chemotherapy group and non chemotherapy group

An independent t- test results showed that mean baseline weight SDS were lower in chemotherapy group and it is statistically significant. However, the difference of mean weight on follow up among chemotherapy group and non chemotherapy group is not statistically significant.

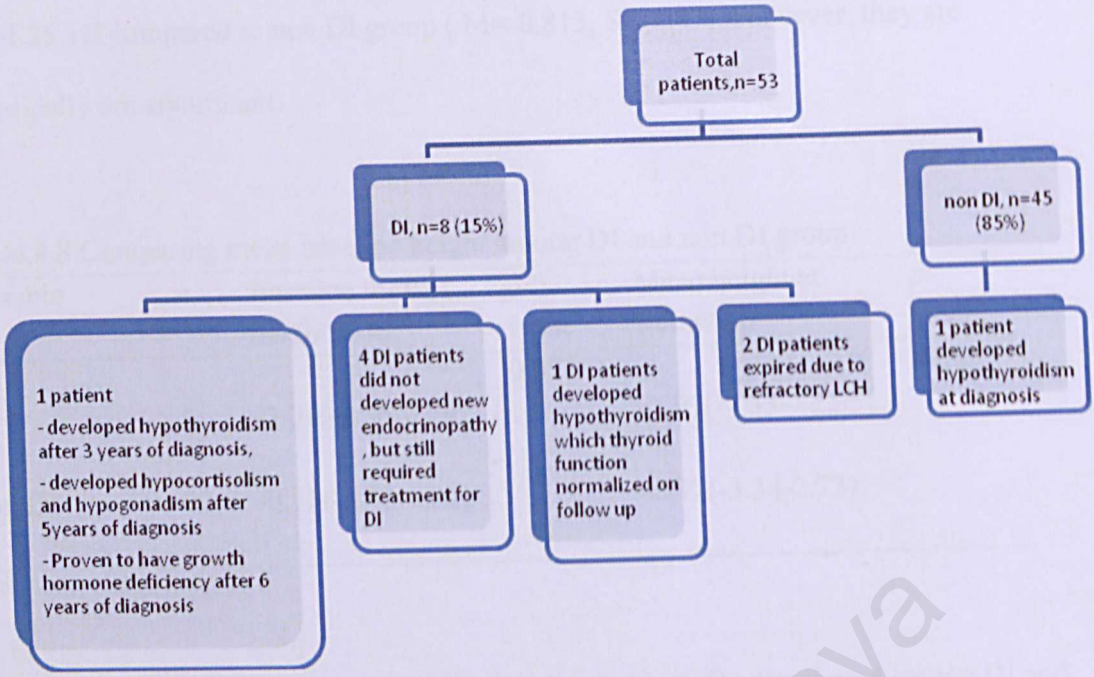

Figure 4.18 Flow chart showing endocrinopathies in LCH patients.

Among 53 patients, no patients had pre-existing diagnosed endocrinal illness.15% of LCH patients developed diabetes insipidus at the beginning of LCH diagnosis. One of the DI patient developed hypothyroidism after 3 years of diagnosis, hypocortisolism and hypogonadism after 5 years of diagnosis and proven to have growth hormone deficiency after 6 years of diagnosis. Another one patient developed subclinical hypothyroidism but the thyroid function subsequently normalized on follow up. Four patients did not developed new endocrinopathy but still required treatment for diabetes insipidus. Two patients passed away due to refractory LCH. Among the non-DI patients, one patient had hypothyroidism as the LCH affected the thyroid gland at diagnosis itself.

We further analyzed the growth of LCH patients based on whether they are having concomitant diabetes insipidus. On inspection of histogram revealed baseline height were normally distributed among DI and non DI group. Therefore, an independent *t*-test was run on the data as well as 95% confidence interval (CI) for mean difference. The results showed that mean baseline height was lower in the DI group (M= -1.632,

SD=1.25) if compared to non DI group (M=-0.813, SD 1.47). However, they are statistically not significant.

Table 4.8 Comparing mean baseline height among DI and non DI group

Variable	*n*	Baseline mean height SDS	*p*-value[a]	Mean height at follow up	*p*-value[a]
			0.074		0.003
DI	6	-2.01(-4.56-0.70)		-2.93(-4.33—2.35)	
Non DI	22	-0.72(-4.32-1.81)		-0.72(-3.34-2.73)	

Note:[a]Independent *t* test

On inspection of histogram revealed weight were normally distributed among DI and non DI group. Therefore, an independent *t*-test was run on the data as well as 95% confidence interval (CI) for mean difference. The results showed that baseline mean weight SDS and mean weight SDS on follow up are lower for DI group. The difference between baseline mean weight SDS is statistically significant among DI and non DI group.

Table 4.9 Comparing mean baseline weight among DI and non DI group

Variable	*n*	Baseline mean weight SDS	*p*-value[a]	Mean weight at follow up	*p*-value[a]
			0.007		0.130
DI	6	-2.62(-4.01—1.84)		-1.99(-4.90-0.67)	
Non DI	22	-0.53(-4.73-1.89)		-0.75(-4.84-1.79)	

Note:[a]Independent *t* test

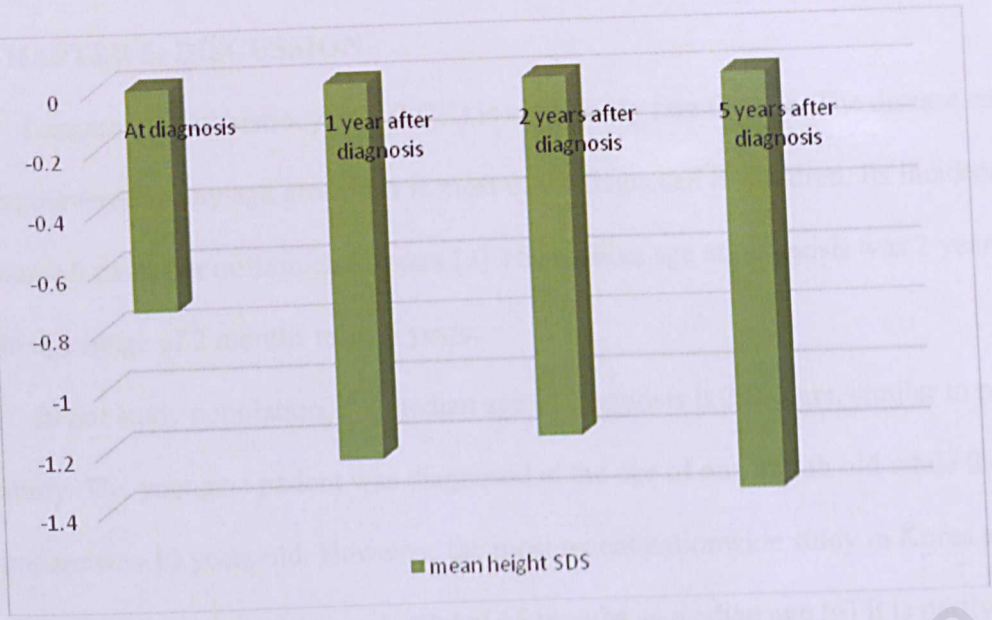

Figure 4.19 Serial mean height SDS of LCH patients with concomitant DI

For the patient with concomitant diabetes insipidus, we further analyzed their serial growth based taking into account of mid-parental height. Among the six DI patient, we noticed they have poor height catch up throughout their follow up.

Figure 4.20 Serial mean weight SDS of LCH patients with concomitant DI

For the six DI patients, though they had poorer height on follow up, they did better in terms of weight gain.

CHAPTER 5: DISCUSSION

Langerhans cell histiocytosis (LCH) is a relatively rare disease. The disease can be encountered in any age group but is most often diagnosed in children. Its incidence rate was 2.6 cases per million child years.[3] The median age at diagnosis was 2 years, with an age range of 2 months to 14.6 years.

In our study population, the median age of diagnosis is 2.0 years, similar to previous study. The youngest patient was diagnosed at the age of one month old while the oldest patient was 10 years old. However, the most recent nationwide study in Korea showed a higher median age at diagnosis, quoted 65 months as median age.[6] It is partly due to the large number of the study subjects with the wide age range reported from 0 months to 276 months of age during diagnosis.

Table 5.1 : Median age of LCH patient

Study	Median Age (years)
Current study	2.0
Sweden[5]	3.8
Argentina[18]	1.5
France[4]	2.8
England[3]	2.0
Malaysia [9]	2.8
South India[7]	3.0
Brazil[8]	2.4
Korea [6]	5.4
United States[19]	1.0

In our study, it was evident that more male (64.2%) were affected compared to female (35.8%). Korean reported male to female ratio as 1.4:1[6] and France reported similar male to female ratio as 1.2:1[4]. The Indian study showed a higher male to

female ratio, 3:1[7]. However, the Brazilian and United States study showed more female being affected. 54 % of female were affected in the Brazilian study and 57.5% of female were affected according to an United States study.

Table 5.2 : Gender Distribution of LCH patient

Study	Male (%)	Female (%)
Current study	34 (64.2%)	19 (35.8%)
Sweden[5]	16(55%)	13(45%)
France [22]	80 (54.4%)	67 (45.6%)
France(French, Cell et al. 2004)	83 (56%)	65 (44%)
England [3]	53 (52.5%)	48 (47.5%)
Malaysia [9]	9 (53%)	8 (47%)
South India[7]	30 (75%)	10 (25%)
Brazil[8]	17 (46%)	20 (54%)
Korea [6]	354 (58.7%)	249 (41.3%)
United States[19]	23 (42.5%)	31 (57.5%)

Majority of children affected are Chinese (43.4%), followed by Malay (41.5%) and Indian (13.2%). Our current observation probably not reflecting our true national distribution of population by ethnicity since this is a single centre study. It may just represent the population of patients who opted to seek treatment in our centre.

Table 5.3 : Risk Stratification of LCH patient

Study	Multisystem	Single system
Current study	33 (62.3%)	20 (37.7%)
Sweden[5]	9 (31%)	20 (69%)
France [22]	108(42.3%)	147 (57.7%)
England [3]	57(56%)	44(44%)
South India [7]	20 (50%)	20 (50 %)
Brazil [8]	20 (54%)	17 (46%)
Korea [6]	184 (30.5%)	419 (69.5%)

In our study, we noticed most of our patients belongs to multisystem group, regardless of whether they have risk organ involvement or not. This finding is supported by the review done in England and Brazil. However, the most recent study done in Korea showed that the single system involvement accounts the majority of their patient population.

Only 42% of our patient had definitive diagnosis which their biopsies demonstrated CD1a positive cells and the rest was considered to have presumptive LCH as their biopsies had characteristic morphology and phenotype recognised and the cells express S100. This is partly due to the availability of the CD1a staining which only commonly used after the year of 2010.

There is a wide variability in clinical manifestation of LCH patient. They may present with clinical presentation involving osseous and extraosseous manifestation. Overall, in our study, LCH mainly affects the bone, similarly shown in most of the literature. The presenting signs and symptoms in our study had mimicked most of the earlier studies. However, liver involvement was found to be high in our center, mainly can be attributed to awareness of screening the liver involvement as we are also a hepatology-gastroenterology center.

Table 5.4 organ involvement comparing LCH studies

Study	Bone	Hematopoietic system	Liver	Spleen	Lymph node	Skin
Current study	37(70%)	11(21%)	21(40%)	10(19%)	14(26%)	12(23%)
Korea[6]	481(80%)	44(7%)	74(12%)	43(7%)	83(14%)	118(20%)
France[4]	191(74%)	13(5%)	11(4%)	11(4%)	NA	86(33%)
England[3]	67(66%)	NA	16(16%)	16(16%)	41(41%)	37(37%)
Sweden[5]	24(83%)	2(7%)	1(3%)	1(3%)	2(7%)	10(34%)
Brazil[8]	25(68%)	NA	9(24%)	7(19%)	17(46%)	17(46%)
India [7]	28(70%)	NA	NA	NA	16(40%)	10(25%)

Table 5.5 : Survival outcome of LCH subjects

Study	Overall survival
Current study	74.7%
Argentina[18]	59% (only confined to multisystem group)
France [4]	99% (1 and 2 year OS)
England [3]	71% (5 year OS)
Brazil[8]	88.5% (5 year OS)
Korea[6]	95.4% (5 year OS)
United States [19]	90% (5 year OS)

When comparing the overall survival outcome, our recent study showed a lower 5-year overall survival if compared to the large population study. International studies sponsored by Histiocyte Society had shown that survival rates for patients with multi-system LCH have progressively improved to as high as 80% for patients with risk organ involvement. [19] Impact of the chemotherapy regime is difficult to be evaluated due to the retrospective nature of this study. In the absence of clear histologic predictors of the natural course of the disease, risk stratification is a good predictor of the outcome. Advances in treatment and supportive care had resulted improvement of the outcome of LCH children. Our study showed excellent survival rate in SS and MS-RO$^-$ group. All subject who died belongs to MS-RO$^+$ group. Poor survival outcome seen in MS-RO$^+$ group does not suggest low efficacy of the chemotherapy regime, but can be attributed

to the poor disease status itself. Young age also shown to be associated with poorer outcome.

Height and weight standard deviation score (SDS) were calculated at diagnosis and at the last review. There are still patient who had not yet attained their final height since they had not completed their growth. 49 patient were included in the analysis at diagnosis as there were 4 missing data. Twelve out of the 49 patients died of their disease. Of the survivor, there were thirteen patients who had lost to follow up. There was no improvement noticed in terms of height SDS after treatment. This observation suggest that the underlying disease per se may responsible for the suboptimal height gain especially those had dramatic growth failure during the active disease. [23]

We further analyzed the growth of LCH subjects based on the 3 risk stratified sub-grouping. Subjects which belongs to multisystem risk organ involvement group had poorer weight and height at baseline and follow up if compared to the other two sub-grouping. This results is supported by a recent Indian population study. [24] Failure to thrive is not frequently documented in children with LCH in the Western literature. Subjects in SS and MS RO$^-$ group are noted to have poorer height gain during their follow up as most of them received steroid as part of the treatment regime.

Diabetes insipidus is the commonest endocrine disorder reported in 12-30 % of LCH subjects. [25] The risk of developing diabetes insipidus was 20% at 15 years after diagnosis.[21] The prevalence of diabetes insipidus reported in the present study is similar to that reported earlier from India(17–25 %). No patient had developed DI after diagnosis. Treatment of DI is usually lifelong but spontaneous resolution of DI has been reported previously in rare instances. None of our subjects showed complete recovery after chemotherapy. Authors noticed adverse effect on growth in subjects with DI, which they had poorer height and weight at baseline as well as during follow up. They did not showed catch up in terms of height during follow up. At diagnosis, DI subjects

had poor weight which could possibly be postulated due to underlying malnutrition, anemia and chronic disease per se. However, they demonstrated acceptable weight increment after treatment commencement likely due to steroid effect which had been known to increase appetite. One of them had been fully investigated and treated as panhypopituitarism and started on growth hormone therapy. The final height of this patient had significantly improved after the commencement of growth hormone. We had one patient presented with solitary thyroid involvement, which is extremely uncommon and is rarely reported in pediatric LCH. [10]

CHAPTER 6 CONCLUSION

In conclusion, patients with young age and risk organ involvement had poorer outcome. Patient with risk organ involvement, concomitant diabetes insipidus and who had underwent chemotherapy also associated with poor height and weight gain during follow up. This study is an insight to the growth and endocrine disorder which can co-exist in children with LCH. Growth monitoring should form an integral part of the LCH management and early referral should be done for subjects with faltering growth. Patients with DI should have early assessment of anterior pituitary function as early growth hormone replacement may improve final stature.

CHAPTER 7 STUDY LIMITATIONS & RECOMMENDATIONS

7.1 Study Limitation

1. Retrospective study. We rely heavily on accurate documentation and record keeping of the patients' case notes.

2. Single center study. This may not reflecting the true picture of patient's characteristic in the whole country, even though we received referral from other states.

3. Small study population. This may affect the validity of the outcome.

7.2 Recommendation

In order to examine the overall situation of LCH children in our country, it would be beneficial to involve other centers in our country which provide paediatric oncology service. Furthermore, I would recommend a prospective study to enable analysis of the other confounding factor which contribute to poor height and weight gain along the treatment period, for example, the diet consumption, awareness and knowledge about dietary modifications, serial growth, height velocity and mid-parental height.

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