MOLECULAR CYTOGENETICS OF MYELODYSPLASTIC SYNDROME – HOSPITAL BASED STUDY

by

ASMIDA BINTI ISA

Thesis submitted in fulfilment of the requirements for the degree of

Master of Science

July 2014

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious and the Most Merciful. Alhamdulillah, all praises to Allah for the strengths and His blessing in completing this thesis. I take this opportunity to express my profound gratitude and deep regards to my supervisor, Professor Dr. Narazah Mohd Yusoff, for her supervision and constant support. You have given me opportunity, advices and trust to execute all the necessary things throughout my study period. All the experiences I had gone through have helped me to grow up and shall be guidance in my journey of life. Also, my appreciation goes to my co-supervisor, Dr Syed Atif Ali for his support, knowledge and constructive comments and suggestions throughout my study period. Your encouragement and engagement during my study are greatly appreciated.

I also take this opportunity to express a deep sense of gratitude to; Dr. Goh Ai Sim, Dr Nabeelah and Dr Chew Teng Kiat from Hospital Pulau Pinang, Prof. Dr Rosline Hassan, Prof. Ravindran Ankathil and Cik Selamah Ghazali from Pusat Pengajian Sains Perubatan, Universiti Sains Malaysia Kubang Kerian who had remarkably helped me in samples collection. And also many thanks to Prof. Jogenananda Pramanik from Alliance University College of Medical Sciences. My wish, I really hope that we can continue to co-operate in the future.

I am obliged to staff members of Advanced Medical and Dental Institute for their help especially En. Abd. Rahman, Cik Faizatul Syima, Pn Ruzzieatul Akma, Pn Fatimah Azlina, Pn Siti Fatimah and all the medical laboratory technologists at

Genetic Lab. Last but not least, my deepest thanks to my friends, Salem, Emmanuel, Abdullah and Omar for their kindness and moral support during my study. Thanks for the friendship and memories. To those who contributed in this research both directly or indirectly, many thanks and may Allah bless you all.

DEDICATION

Special thanks are dedicated to my husband, Ruslan for his support, encouragement and prayer. To my loving parents, Mr Isa Ahmad and Mrs Mik Som Abdullah, thank you so much for your love and prayers.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

AML	Acute Myeloid Leukaemia
Array-CGH	Comparative Genomic Hybridization
BAC/PAC	Bacterial /Plasmid Artificial Chromosome
BMA	Bone Marrow Aspiration
CDR	Commonly Deleted Region
CGH	Comparative Genomic Hybridization
CK	Complex karyotypes
CML	Chronic Myeloid Leukaemia
CMML	Chronic Myelomonocytic Leukemia
CNV	Copy Number Variants
Del/del	Deletion
DLCL	Diffuse Large-Cell Lymphoma
dsDNA	Double Stranded DNA
FAB	French American British
FBP	Full Blood Picture
FISH	Fluorescent In-situ hybridization
FISH	Fluorescent In-Situ Hybridization
gDNA	Genomic DNA
HgB	Hemoglobin
ICD-10	International Classification of Diseases-10
IPSS	International Prognostic Scoring System
MDS	Myelodysplastic syndrome
MDS-U	MDS Unclassifiable
MOH	Ministry of Health
N:C	Nucleus:Chromatin
OD	Optical Density
OS	Overall Survival
RA	Refractory Anaemia
RAEB	Refractory Anaemia With Excess Blasts
RAEB-1	Refractory anaemia with excess blasts-1

RAEB-2	Refractory anaemia with excess blasts-2
RAEB-T	Refractory Anaemia With Excess Blasts In Transformation
RARS	Refractory Anaemia With Ring Sideroblasts
RBC	Red Blood Cells
RCMD	Refractory Cytopaemias With MultilineageDysplasia
RCUD	Refractory Cytopaenias With Unilineage Dysplasia
RN	Refractory Neutropaenia
RT	Refractory Thrombocytopaenia
WBC	White Blood Cells
WHO	World Health Organization

LIST OF SYMBOL

-	Deletion
+	Trisomy
del	Deletion
inv	Inversion
t	Translocation

SITOGENETIK MOLEKULAR MIELODISPLASTIK SINDROM

- KAJIAN BERASASKAN HOSPITAL

ABSTRAK

Mielodisplastik Sindrom (MDS) adalah satu keadaan haematologi yang melibatkan sel-sel di dalam sum-sum tulang yang tinggi dan kiraan kandungan darah yang rendah. Tujuan kajian ini dijalankan adalah untuk mengenalpasti ketidaknormalan kromosom pada pesakit MDS menggunakan teknik sitogenetik dan "array-comparative genomic hybridization" ("array-CGH") dan mengaitkannya dengan penemuan hematologi. Sejumlah 8 pesakit telah diambil untuk kajian ini. Keputusan sitogenetik menunjukkan 6 kariotaip normal, satu kariotaip ketidaknormalan struktur (del(5)(q13q33) dan satu kariotaip ketidaknormalan kompleks (t(1;11), t(2;11), del(4p), del(5p), del(9p)). Menggunakan teknik "array-CGH", ketidaknormalan yang diketahui, kompleks dan jarang berlaku/"cryptic" (<5 Mb) telah dikesan. Dalam kes ketidaknormalan yang diketahui, del5q (q13.1 - q13.2 (2.4 Mb)) dan amplifikasi besar pada kromosom 8 (p23.3-p11.1 (46.3 Mb) dan q11.1-q24.3 (98.7 Mb)) telah dikesan. Dalam ketidaknormalan kompleks, ketidaknormalan melibatkan saiz 0.7 Mb ke 98.7 Mb. Tiga pesakit telah menunjukkan persamaan dalam penemuan haematologi. Lima ketidaknormalan "cryptic" dikenalpasti dalam 3 pesakit (0.7 Mb ke 4.8 Mb). Secara rumusan, ketidaknormalan diketahui, kompleks dan jarang berlaku/"cryptic" telah dikesan. Perkaitan penemuan haematologinya boleh memberikan maklumat untuk menyediakan rawatan yang sesuai kepada pesakit. Penemuan ini juga boleh menyumbang kepada kepelbagaian ketidaknormalan kromosom di kalangan pesakit MDS.

MOLECULAR CYTOGENETICS OF MYELODYSPLASTIC SYNDROME – HOSPITAL BASED STUDY

ABSTRACT

Myelodysplastic Syndrome (MDS) is a haematological condition characterised by hypercellular bone marrow and low peripheral blood counts. The objectives of this study were to identify chromosomal aberration using conventional cytogenetics and array comparative genomic hybridisation (array-CGH) in MDS patients and correlate with its' haematological findings. A total of 8 patients were recruited. Cytogenetic results revealed six normal karyotypes, a karyotype with structural abnormalities (del(5)(q13q33) and a karyotype with complex rearrangement (t(1;11), t(2;11), del(4p), del(5p), del(9p)). By array-CGH technique, known cytogenetic aberrations, complex chromosome aberrations and rare/cryptic aberrations (<5 Mb) were detected. In the case of common aberrations, del5q (q13.1 - q13.2 (2.4 Mb)) and large amplification of chromosome 8 (p23.3-p11.1 (46.3 Mb) and q11.1-q24.3 (98.7 Mb)) were detected. In complex aberrations, the aberrations involved from 0.7 Mb to 98.7 Mb in size. These patients showed similarities of haematological findings. Five cryptic aberrations were identified in 3 patients (0.7 Mb to 4.8 Mb). As a conclusion, common, complex and cryptic aberrations were detected in MDS patients. The associated haematological findings could provide useful information to stratify the suitability of the treatments for the patients. These findings also might contribute to the heterogeneity of choromosomal aberrations in MDS patients.

CHAPTER 1

INTRODUCTION

1.1 Background

Myelodysplastic Syndrome (MDS) is a haematological medical condition that involves ineffective production of the myeloid cells. It is characterised by progressive reduction in myeloid cells in the peripheral blood, reflecting defects in red blood cells (RBC), white blood cells (WBC) or platelets maturation.

Myelodysplastic Syndrome (MDS) are much more common in elderly. The incidence of MDS varies by population, 3.5–12.6 per 100 000 population per year with 15-89 per 100, 000 in elderly per year (Aul *et al*., 1998; Sole *et al*., 2000; Cogle *et al*., 2011; Guillermo *et al.*, 2011). MDS rarely affect individuals younger than age 50, unless preceeded by chemotherapy or radiation for another malignancy.

The overall incidence of MDS is slightly higher in males than in females (1.5 to 2.1). In people over 70 years of age, incidence rates are about 20 to 40 per 100, 000 population, suggesting that MDS is at least as common as chronic lymphocytic leukaemia or multiple myeloma (Ulrich *et al*., 2004).

There are two categories of MDS; de novo MDS and secondary MDS. In *de novo* MDS, a transforming event affects a pluripotent or multipotent progenitor cell in the

bone marrow, conferring a growth advantage upon it and eventually establishing clonal haematopoiesis.

However, secondary MDS is "therapy-related MDS/AML" following exposure to alkylating agents is the only clear etiological factor thus identified. Increasing evidence for exposure to benzene and radiation and the development of MDS is emerging (Pagano *et al.*, 2006). Benzene haematotoxicity is mediated via both genotoxic and non-genotoxic mechanisms, leading to aplasia, apoptosis and initiation (via genetic mutation) of clonal disorders such as MDS (Rinsky *et al.,* 1987).

Many patients in the early stages of MDS experience no symptoms at all. A routine blood test may reveal a reduced red cell count (anaemia), sometimes along with reduced white cell (leukopaenia) and/or platelet counts (thrombocytopaenia). On occasion, the white cell and platelet counts may be low while the red cell count remains normal. In the early stages of this disease, the counts are usually not so reduced that they produce symptoms. However, some patients, particularly those with blood cell counts well below normal, experience definite symptoms (Mary, 2005).

Anaemic patients generally experience fatigue and report that they are tired much of the time and have no energy. About 80% (Peter & Bennet, 2006) of patients are anaemic when they are initially diagnosed with MDS. Anaemia varies in its severity. In mild anaemia, patients may feel well or just slightly fatigued. In moderate anaemia, almost all patients experience some fatigue, which may be accompanied by heart palpitations, shortness of breath, and pale skin. In severe anaemia, almost all

patients appear pale and suffer severe fatigue and shortness of breath. Because severe anaemia reduces blood flow to the heart, older patients may be more likely to experience cardiovascular symptoms, including chest pain (Guillermo *et al*., 2011).

A reduced white cell count lowers the body's resistance to bacterial infection. Patients with neutropaenia may be susceptible to skin infections, sinus infections, lung infections or urinary tract infections. Fever may accompany these infections (Mary, 2005).

Patients with low platelets count have an increased tendency to bruise and bleed even after minor bumps and scrapes. Bruises can be dramatic, some as large as the palm of the hand (Mary, 2005).

Recently, the classification of this disease has been refined to include new scientific and clinical information – including cytogenetics. Based on the studies carried out, cytogenetic abnormalities have been observed in 40-50% of patients with MDS (Athena *et al*., 2003; James *et al*., 2009) and the karyotype may exhibit differences between primary (*de novo*) and secondary MDS.

The disease course varies widely from patient to patient, from indolent to aggressive with swift progression to acute myeloid leukaemia (AML) in 30% of cases (Besa *et al*., 2009). Patients with favourable prognoses are often associated with normal karyotype or a single chromosomal abnormality.

The prognosis becomes worse when it is associated with complex karyotype (>3 abnormalities). It is found that, a strong association between numbers of abnormalities with poor prognostic value in patients with primary MDS (David *et al*., 2013). The results also included shorter survival rate and high risk progression toward AML.

Studies also showed that occurrence of rare cytogenetic abnormalities have a relatively grim clinical outcome. For example, Wang *et al.* (2010) suggested that rare occurrence of trisomy 11 in MDS is best considered as a high-risk cytogenetic abnormality in MDS prognostication due to its clinical aggressiveness towards evolving AML with myelodysplasia-related changes. Although various chromosomal abnormalities have been identified in patients with MDS, the specific genes that play roles in the disease development are yet to be identified (Bernadette *et al*., 2007).

1.2 Justification of the Study

A significant portion of MDS patients have chromosomal abnormalities. Although wide researches on MDS have been carried out, the prevalence of local MDS data is not available. Several case reports and a single scientific study have been reported before (Marini *et al*., 2005; Yin *et al*., 2011; Zainina *et al.,* 2006; Mohadese *et al*., 2012).

Reference to Health Informatics Center, Ministry of Health (MOH) found that the data is grouped together under D37-D48 "Neoplasm of uncertain or unknown behaviour" based on International Classification of Diseases-10 (ICD-10) which has made it difficult to categorise the exact incidence of this disorder. Additionally, research on it has not been done extensively. Based on a haematological study carried out at Hospital Kepala Batas/Advanced Medical and Dental Institute from November 2003 to December 2007, only 2 cases were confirmed as MDS from 3804 Peripheral Blood Film (PBF) reviewed. The cases were confirmed by Peripheral Blood Film (PBF) & Bone Marrow Aspiration and Trephine Biopsy (BMAT). The objective of this was to obtain the profiles and clinical features of MDS (Lee *et al.,* 2009).

Therefore, in order to understand the cause of this disorder comprehensively, this study was carried out to investigate the findings of chromosomal aberrations in patients with MDS using available cytogenetics technique i.e conventional cytogenetics and array-based Comparative Genomic Hybridisation (array-CGH).

1.3 Hypothesis

Many chromosomal aberrations have been associated with the development of MDS. Therefore, it is hypothesised that the chromosomal aberrations in patients with MDS using conventional cytogenetics techniques and array-CGH techniques will be clarified.

1.4General Objective

The general objective of this study was to investigate chromosomal aberrations in patients with MDS with its associated haematological findings.

1.5 Specific Objectives

1. To identify common and cryptic aberrations in patients with Myelodysplastic Syndrome (MDS) using conventional cytogenetic and array-based Comparative Genomic Hybridisation (array-CGH) techniques.
2. To associate haematological and clinical findings and molecular cytogenetics in patients with MDS.

1.6 Literature Review

1.6.1 Myelodysplastic Syndrome (MDS)

Myelodysplastic syndrome (MDS) is a group of conditions in which there is disordered maturation (dysplasia) in one or more of the myeloid cell lineage. Blood cell production from the dysplastic cells is ineffective and therefore, may lead to anaemia, thrombocytopaenia or neutropaenia.

The population of dysplastic cells represents an abnormal clone in the stem cell which is stable for a variable period (sometimes for several years). It may generate new clones associated with increasingly ineffective haematopoiesis or increasing dysfunction of mature cells (Bernadette *et al.,* 2007). The majority of the patients are elderly with many abnormalities that may be seen on the blood film; often only a single abnormality may be found at diagnosis and others appear later. Some patients are diagnosed incidentally as a consequence of having a blood count for unrelated reason. Others present with symptoms referable to anaemia, thrombocytopaenia or neutropaenia and to functional abnormalities of neutrophils and platelets (Mary, 2005).

The signs and symptoms of MDS result from the infiltration of the blasts into bone marrow and peripheral blood. A history of infections, bleeding, weight loss or cardiovascular symptoms may be the complaints made by the patients. Signs of anaemia are pale skin and easy fatigability. Infections are caused by reduction of

white blood cells (WBC) and thrombocytopaenia may be a feature as manifested by gum bleeding and easy bruising (Bernadette *et al*., 2007).

Clonal disorders in MDS has been proposed to progress through the stages of tumorigenesis, in which the unstable haematopoietic stem cells are susceptible to genetic lesion. Once a stem cell gains a dominant mutation over the normal cell growth, the cell shows clonal evolution to become much more susceptible to further multiple genetic mutations. At this stage it is most likely accompanied by a high rate of cell death. Finally, it will lead to malignant transformation with an increase in leukemic blast cells and evolution to acute myeloid leukaemia (Aul *et al*., 1998; Hisamaru, 2003). Though, the initial event that causes this primary lesion remains unknown.

1.6.2 Classification of MDS

At present, MDS is classified according to the two most common systems – French American British (FAB) and World Health Organisation (WHO).

FAB scheme divided MDS into five subgroups, based on percentage of blast cells in the peripheral blood and bone marrow, the presence of ringed sideroblasts in the bone marrow and the monocyte count in the peripheral blood (**Table 1.1**) (Bennet *et al.,* 1982).

Based on biology, immunophenotyping, genetic and clinical features, WHO classified MDS in the following seven groups: refractory cytopaenias with unilineage dysplasia (RCUD), refractory anaemia with ring sideroblasts (RARS), refractory anaemia with multilineage dysplasia (RCMD), refractory anaemia with excess blasts-1 (RAEB-1), refractory anaemia with excess blasts-2 (RAEB-2), MDS, unclassifiable (MDS-U) and MDS associated with isolated del(5q) (James *et al.,* 2009).

Although there are many differences between adult and paediatric MDS, neither FAB nor WHO have used age in their classification. The three significant differences between FAB and WHO classifications are noted here. First, WHO lowered the percentage of blasts required for MDS diagnosis to at least 20% blasts instead of 30% as recorded in FAB. Second, the inclusion of multilineage dysplasia subtype in the cases of patients with cytopaenia. And third, the addition of MDS with isolated del(5q) which is lacking in FAB. WHO seems to be more clinically useful than FAB

classification as the system recommends using all available data including biologic, immunophenotypic, genetic and clinical features for a proper identification.

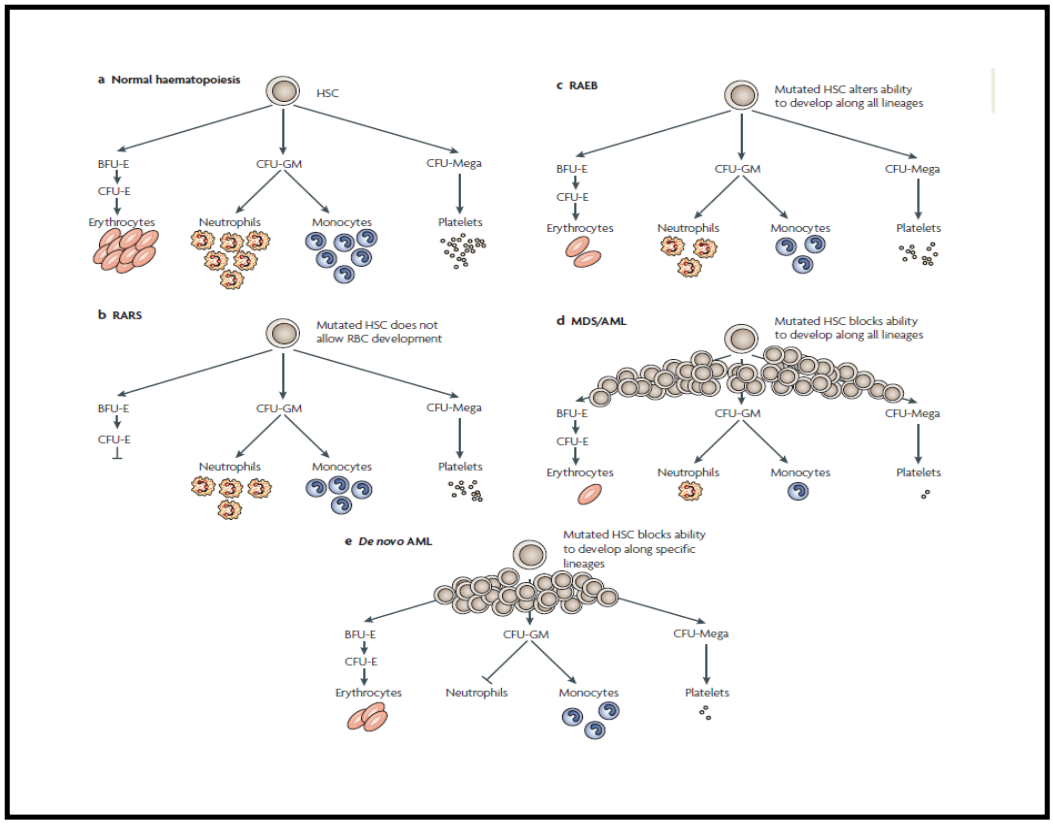

Figure 1.1. MDS constitute a complex range of stem-cell diseases. The myelodysplastic syndromes (MDS) cell clone can suppress normal haematopoiesis (a) directly or indirectly through stroma. Stem-cell defects can result in single lineage deficiency (refractory anaemia and ringed sideroblasts (RARS; **b**)) or multiple-lineage deficiencies (refractory anaemia with excess blasts (RAEB; **c**)). MDS stem-cell diseases (**d**) might seem like de novo acute myeloid leukaemia (AML), however, the two are distinguishable. For example (**e**), cytopaenias in *de novo* AML can be more restricted owing to a failure in differentiation. HSC: haematopoietic stem cell (Reproduced from James *et al.*, 2009).

Table 1.1.FAB classification of MDS (Reproduced from Bennet *et al*., 1982).

Category	Dysplasia	% BM Blasts	%PB Blasts
Refractory anaemia (RA)	Erythroid	<5	<1
Refractory anaemia with ring sideroblasts (RARS)	Erythroid	<5	<1
Refractory anaemia with excess blasts (RAEB)	2 or more lineages	5-20	0-4
Refractory anaemia with excess blasts in transformation (RAEB-T)	Usually 2 or more lineages	21-30	$\geq$5
Chronic myelomonocytic leukaemia (CMML)	Variable $\geq 1 \times 10^9$/L monocytes	<20	$\geq$5

Table 1.2.WHO classification of MDS (Reproduced from James *et al,* 2009*)*.

Disease entity	Blood findings	Bone marrow findings
Refractory cytopaenias with unilineage dysplasia (RCUD): refractory anaemia (RA), refractory neutropaenia (RN), refractory thrombocytopaenia (RT)	Unicytopaenia or bicytopaenia[a] No or rare blasts (<1%)[b]	Unilineage dysplasia: ≥10% of the cells in one myeloid lineage , <5% blasts, 15% of erythroid precursors are ring sideroblasts
Refractory anaemia with ring sideroblasts (RARS)	Anaemia No blasts	≥15% of the erythroid precursors are ring sideroblasts. Dyserythropoiesis only <5% blasts
Refractory anaemia with multilineage dysplasia (RCMD)	Cytopaenia(s), no or rare blasts[b] No Auer rods <1 $\times 10^9$/L monocytes	Dysplasia in >10% of cells in 2 or more lineages <5% blasts in marrow No Auer rods <1 $\times 10^9$/L monocytes
Refractory anaemia with excess blasts-1 (RAEB-1)	Cytopaenia(s) <5% blasts No Auer rods <1 $\times 10^9$/L monocytes	Unilineage or multilineage dysplasia 5–9% blasts No Auer rods
Refractory anaemia with excess blasts-2 (RAEB-2)	Cytopaenia(s) 5–19% blasts Auer rods ±[c] <1 $\times 10^9$/L monocytes	Unilineage or multilineage dysplasia 0–19% blasts Auer rods ±[c]
MDS, unclassifiable (MDS-U)	Cytopaenias <1% blasts[b]	Unequivocal dysplasia in less than 10% of cells in one or more myeloid lines when accompanied by a cytogenetic abnormality considered as presumptive evidence for a diagnosis of MDS <5% blasts

MDS associated with isolated del(5q)	Anaemia Usually normal to elevated platelets No or rare blasts	Normal to increased megakaryocytes with hypolobulated nuclei <5% blasts del(5q) is the sole cytogenetic abnormality No Auer rods

[a]Bicytopaenia may be occasionally observed. Cases with pancytopenia should be classified as MDS-U.
[b] If marrow blast % is less than 5%, but there are 2–4% myeloblasts in blood, the diagnosis is RAEB-1. Cases of RCUD and RCMD with 1% blasts in blood are classified as MDS-U.
[c] Cases with Auer rods and <5% myeloblasts in the blood and <10% in the marrow should be classified as RAEB-2.

1.6.3 Cytogenetic Abnormalities

Genetic aberrations, as losses of genetic material (deletions) or localised gains that affect certain region of the genome have been shown to be the basis of many diseases. Occurrence of one or more of genetic alterations in the genome that lead to changes in DNA sequence copy number have been well documented to characterise the prevalence of rare and common diseases.

In MDS, it has been observed cytogenetic abnormalities in 40 - 50% of patients with MDS and the karyotypes may differ between primary (*de novo*) and secondary MDS (Athena *et al.,* 2003; James *et al*., 2009). The differences may be observed on initial bone marrow observation or during evolution of the disease (James *et al*., 2009).

Frequent chromosome abnormalities that have been observed in studies were del(5q), monosomy 7/del(7q), trisomy 8, del(20q) and complex karyotypes (CK) ($\geq$3 abnormalities) (Hisamaru, 2002; Martı´nez-Ramı´rez *et al*., 2004; Look 2005; Martı´nez-Ramı´rez *et al.*, 2005). According to International Prognostic Scoring System (IPSS), the prognosis of these recurrence cytogenetic abnormalities is described here: del(5q) and del(20q) - good prognosis; del(7q) and trisomy 8 - intermediate prognosis, monosomy 7 - poor prognosis and CK – very poor prognosis. CK also have the highest risk to evolve to AML.

Del(5q) and monosomy 7/del(7q) occur in about 20% of MDS patients and the incidence increased to 50% in secondary MDS (Cordoba *et al.* 2012; Hisamaru, 2003; West *et al.,* 2000). This is due to these patients have been exposed to benzene.

Benzene derivative metabolite has been proven to induce deletion of chromosome 5 and 7 in human $CD34^{+}CD19^{-}$ bone marrow cells (Stillman *et al.,* 1999; Pagano *et al*., 2006). CK also has shown high incidence in secondary MDS at about 20% incidences (West *et al.,* 2000). Although many chromosomal abnormalities have been identified in patients with MDS, the genes involved in the disease development are yet to be identified (Bernadette *et al*., 2007).

Table 1.3. IPSS Cytogenetic risk groups (Greenberg *et al.*, 2012).

Prognosis	Cytogenetic abnormalities
Very good	-Y, del(11q)
Good	Normal, del(5q), del(12p), del(20q), double including del(5q)
Intermediate	del(7q), +8, +19, inv(17q), any other single or double independent clones
Poor	-7, inv(3)/t(3q)/del(3q), double including -7/del(7q), Complex: 3 abnormalities
Very poor	Complex: >3 abnormalities

1.6.3.1 The 5q- Syndrome

The 5q- abnormality is the most common abnormality found and constituted of more than 20% in MDS patients (Hisamaru, 2003). The diagnosis was restricted to the cases of isolated interstitial del(5q), without excess blasts in the bone marrow (<5%) (James *et al*., 2009). It was first reported as a type of refractory anaemia with clinical features; female predominance (unlike other MDS), macrocytosis, erythroid hypoplasia, frequent thrombocytosis and dysmegakaryopoiesis (Van den *et al*., 1985). A summary of the clinical and haematologic features that are specific to the 5q- syndrome subtype of MDS is shown in **Figure 1.2**.

The breakpoints within a large region of 5q are highly variable among patients, but the most critical region of deletion is supposed to lie between 5q13 and 5q33 (Kanehira *et al*., 2009, Aristoteles *et al*., 2006). Several genes encoding haemopoietic growth factors and receptors, comprising *IL-3, IL-4, IL-5, M-CSF* (*CSF-I*), *GM-CSF*, and the receptor for *M-CSF* (*CSF-IR*), are localised to the long arm of chromosome 5. It is believed that deletion of one or more of these genes may be critical to the pathogenesis of the associated MDS (Boultwood, 2000).

By the International Prognostic Scoring System, patients with isolated deletion 5q have a relatively good prognosis with low risk of transformation to AML and are assigned to the low-risk MDS category. Giagounidis *et al.* (2004) reported that a median overall survival of 107 months at a median follow-up of 53 months, and a low probability of transformation to acute myeloid leukaemia These patients typically have an isolated 5q deletion and a medullary blast count <5%.

Despite the low risk of transformation to AML in these patients, the dependence on RBC transfusions often has a negative effect on morbidity and mortality (Boultwood *et al.*, 2000; Giagounidis *et al.*, 2004). Moreover, patients with deletion 5q show excellent response rates when treated with the immunomodulatory drug lenalidomide (List *et al.,* 2006). However, it is thought that the presence of additional chromosomal abnormalities and/or high medullary blast percentage in patients with a 5q deletion can have a negative effect on disease progression and survival.

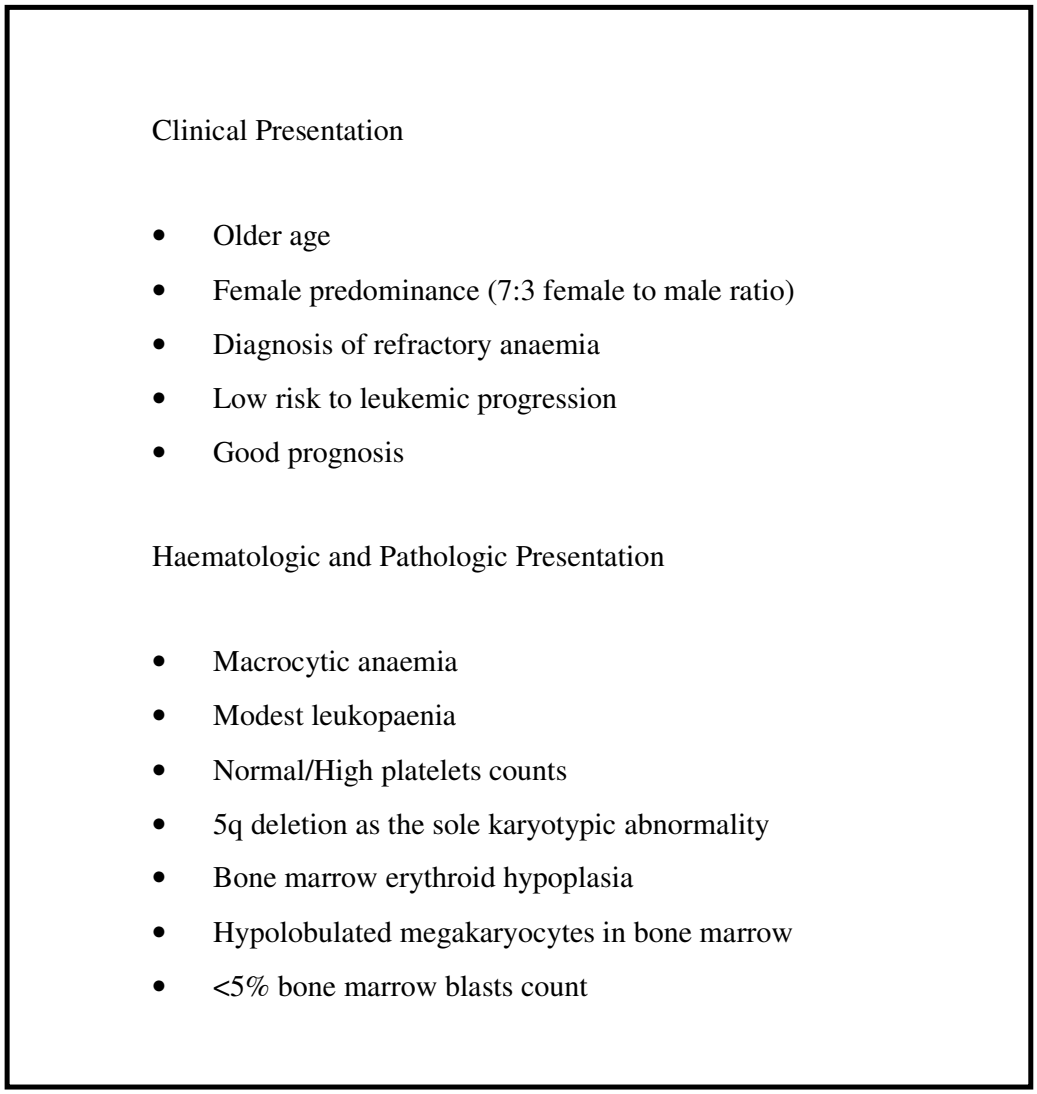

Figure 1.2. Clinical and pathologic characteristic of 5q-syndrome (Adapted from Aristoteles *et al.*, 2006).

1.6.3.2 Monosomy 7 or -7q

Monosomy 7 and -7q are among the most frequent chromosomal abnormalities in MDS. It is associated with poor prognosis in terms of survival or leukaemic evolution. This abnormality may occur *de novo* or be secondary to an exposure to chemical mutagens prior to chemotherapy treatments with alkylating agents. In IPSS, monosomy 7 or -7q is classified as poor prognostic criteria, characterised by infectious susceptibility, quick aggravation and treatment resistance. Abnormalities involving chromosome 7 occur in approximately 20% of patients with MDS and clonal cytogenetic abnormalities and usually include deletion of part of the long arm of chromosome 7 (-7q), total loss of chromosome 7 (monosomy 7), or translocations involving chromosome 7.

Reports have argued that patients who have -7q as a single abnormality overall tend to have a better prognosis than those who have isolated monosomy 7 (Haase *et al.,* 2007; Pozdnyakova *et al.,* 2008). These findings tend to suggest that patients who have MDS with isolated -7q should not be classified in the same group as those who have MDS with monosomy 7. However, those studies involved a very limited number of patients with MDS and chromosome 7 anomalies, and the observed differences in survival between isolated del(7q) and single monosomy 7 were not statistically significant.

Given the high heterogeneity of the break points on 7q, it is suggested that multiple genes are involved in the pathogenesis of myeloid disorder (Brezinova *et al,*. 2007). Although genes on chromosome 7 that are responsible for the disease phenotype are

not yet been identified, a critical region at locus 7q22.1 has been suggested to have significant role in myeloid malignancies (Johnson *et al*., 1996). Cordoba's *et al.* (2012) study also confirmed that 7q22 was the most commonly deleted region in patients with isolated del7q. A potential myeloid tumor suppressor gene, *PIK3CG,* has been identified in this locus, but is unlikely to act as a recessive tumour-suppressor gene in MDS with monosomy 7 (Kratz *et al.,* 2002).

MDS with chromosome 7 abnormalities, have also been shown to associate with somatic mutations of the *RUNX1* gene, and so far monosomy 7 and der(1;7)(q10;p10) have been regarded as similar cytogenetic entities because they both result in loss of 7q. However, in recent study by Westman & Mette (2013) has shown that the other gene that was significantly asscociated with this subgroup. *IDH,* has shown to have mutation up to 37% of the cases with der(1;7)(q10;p10) whereas in monosomy 7, mutations of *RUNX1* and *DNMT3A* genes were equally distributed between the two subgroups (Westman & Mette, 2013). This study was in line with recent findings of different clinical outcomes for patients with der(1;7) and monosomy 7/-7q (Ganster *et al.,* 2013).

In paediatric cases, MDS with monosomy 7 or -7q was reported in a few occasions associated with poor prognosis and progression to acute myeloid leukaemia as well as acute lymphoid leukaemia (Aktas & Tuncbilek, 2006). Recently, it was demonstrated that patients who had MDS with isolated -7q had some distinct clinical-pathologic characteristics as well as better survival than patients who had MDS with monosomy 7 (Cordoba *et al*., 2012).

1.6.3.3 Trisomy 8 (+8)

According to the IPSS, sole +8 is categorised as intermediate cytogenetic subgroup. But as some MDS patients with +8 perhaps progress quickly to acute leukaemia and have shorter survival (Ma *et al*., 2012), some reports have suggested that +8 should be categorised into poor risk cytogenetic group. Although trisomy 8 as the sole chromosome aberration is the most common numerical abnormality in acute myeloid malignancies, little is known about its pathogenic effects. In a study by Paulsson *et al* (2006), who performed high-resolution genome-wide array comparative genomic hybridisation (array-CGH) in MDS cases with trisomy 8 as the sole cytogenetic aberration, have revealed that +8 is not always the primary genetic event and suggested that +8 is not sufficient for leukaemogenesis.

1.6.3.4 Rare Occurrence Chromosomal Abnormalities

Rare occurrences of cytogenetic abnormalities have been observed in numerous studies. A study detected cryptic aberrations of 12p-, 13q-, +21 and +11 in 32 primary MDS patients (Rolf *et al*., 2002). Another study found chromosomal anomalies of del(12)(p12p13), involvement of 13q and del(20)(q11q12) (Sendi*et al.* 2002). Other rarer abnormalities detected were abnormalities in chromosome 3 using conventional cytogenetics (Pozdnyakova *et al*., 2008). Pozdnyakova *et al.* (2008) also reported that +11 as the sole cytogenetics anomaly whose patients died within 21 months after the diagnosis. Other abnormalities found in this study were del(12p), del(11)(q23), and +19. In case of +11, a study by Wang *et al*. (2010) has suggested a strong correlation between +11 and early/evolving AML.

As described above, one complicating factor in the diagnosis of MDS is the heterogeneity of cytogenetic abnormalities. Unlike chronic myeloid leukaemia for example, in which there is one dominant cytogenetic abnormality, t(9;22), numerous cytogenetic abnormalities are associated with MDS. Even though all of these findings were relatively rare, these cytogenetic abnormalities cannot be looked upon lightly due to its different interpretation of its clinical aggressiveness. Accurate, timely diagnosis is important because of the risk of transformation to AML. Overall, the prognosis is poor for patients with MDS, with three-year survival rates estimated to be <50% (Rollison & List, 2004).

1.6.4 Methods of Detection

There are several methods to detect chromosomal abnormalities in MDS patients i.e. cytogenetics, comparative genomic hybridisation (CGH) and array-comparative genomic hybridisation (array-CGH).

1.6.4.1 Cytogenetics Analysis

Cytogenetics is the most widely used method for the identification of chromosome aberrations. Cytogenetics involves karyotyping which organises all chromosomes by homology, size and shape; providing an overview of the whole genome and detects both numerical and structural chromosomal aberrations (overall resolution is 10 Mega bases (Mb); breakpoint resolution is 5 to 15 Mb) (Steven & Martha, 2006).