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<td><strong>Author(s)</strong></td>
<td>Lim, Alvin Soon Tiong; Lim, Tse Hui; See, Karen Hsu Shien; Ng, Yit Jun; Tan, Yu Min; Choo, Natasha Swee Lian; Lim, Sherry Xin Er; Yee, Yenny; Lau, Lai Ching; Tien, Sim Leng; Sathish, Kumar; Tan, Daryl Chen Lung</td>
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Original article

Cytogenetic and molecular aberrations of multiple myeloma patients: a single-center study in Singapore

LIM Alvin Soon Tiong, LIM Tse Hui, SEE Karen Hsu Shien, NG Yit Jun, TAN Yu Min, CHOO Natasha Swee Lian, LIM Sherry Xin Er, YEE Yenny, LAU Lai Ching, TIEN Sim Leng, Kumar Sathish, and TAN Daryl Chen Lung

Keywords: amp(1q21); del(13q); fluorescence in situ hybridization; immunoglobulin heavy chain; Southeast Asia

Background Much is known about the cytogenetic lesions that characterize multiple myeloma (MM) patients from the USA, Europe, and East Asia. However, little has been published about the disease among Southeast Asians. The aim of this study was to determine the chromosomal abnormalities of MM patients in our Singapore population.

Methods Forty-five newly-diagnosed, morphologically confirmed patients comprising 18 males and 27 females, aged 46-84 years (median 65 years) were investigated by karyotyping and fluorescence in situ hybridization (FISH). FISH employing standard panel probes and 1p36/1q21 and 6q21/15q22 probes was performed on diagnostic bone marrow samples.

Results Thirty-four cases (75.6%) had karyotypic abnormalities. Including FISH, a total detection rate of 91.1% was attained. Numerical and complex structural aberrations were common to both hyperdiploid and non-hyperdiploid patients. Numerical gains of several recurring chromosomes were frequent among hyperdiploid patients while structural rearrangements of several chromosomes including 8q24.1 and 14q32 characterized non-hyperdiploid patients. With FISH, immunoglobulin heavy chain (IGH) gene rearrangements, especially fibroblast growth factor receptor 3 (FGFR3)/IGH and RB1 deletion/monosomy 13 were the most common abnormalities (43.4%). Amplification 1q21 was 10 times more frequent (42.5%) than del(1p36) and del(6q21).

Conclusions We have successfully reported the comprehensive cytogenetic profiling of a cohort of newly-diagnosed myeloma patients in our population. This study indicates that the genetic and cytogenetic abnormalities, and their frequencies, in our study group are generally similar to other populations.

Much attention has been paid to elucidate the genetic lesions in multiple myeloma. This arose because of the realization that such lesions provide valuable prognostic information in terms of anticipated response to therapy and survival outcomes. Classical cytogenetics has for many years provided such important information. However, owing to the low mitotic activity of malignant plasma cells in culture, the abnormality pick up rate has been less than satisfactory with the majority of laboratories reporting a 30%–45% detection rate despite the fact that most, if not all, myeloma cells should harbor genetic aberrations. To mitigate the limitations of karyotyping, interphase fluorescence in situ hybridization (FISH) assays have been employed with great success in conjunction with classical cytogenetics with a consequent quantum increase in the detection rate to over 80%. Furthermore, FISH has also enabled the detection of chromosomally cryptic rearrangements that confer a poor prognosis, including fibroblast growth factor receptor 3 (FGFR3)/immunoglobulin heavy chain (IGH) and IGH/MAF fusions. More recently, amplification 1q21 (amp1q21) is also believed to be associated with a poor prognosis, as are deletions of 1p21 and 6q21.

A great deal of genetic basis of multiple myeloma, the good and bad prognosticators and their frequencies, has come from publications based on Western, and more recently, East Asian populations. However, there is a great paucity in the literature about the chromosomal abnormalities that characterize patients of Southeast Asian descent.

Therefore, this study sought to examine the chromosomal and genetic abnormalities from a small series of multiple myeloma patients in Singapore. In addition to determining the prevalence of specific gene rearrangements from well-established FISH panels, this study undertook to examine the incidences of amp1q21, deletion 1p36, deletion 6q21, and gains of chromosome 15 (amp15q22) from the study sample.

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This study was supported by the SingHealth Foundation Grant SHF/FG426P/2009.
METHODS

Patients
Forty-five consecutive newly-diagnosed patients with multiple myeloma were entered into the study. The criteria for the diagnosis of multiple myeloma was based on the International Myeloma Working Group definition. In addition, all cases were morphologically confirmed by bone marrow aspirate morphology and flow cytometry.

The patients included 18 males and 27 females between the ages of 46–84 years (median age 65 years). The retrospective period of recruitment was from January 2007 to November 2010. Diagnostic marrow samples were obtained and processed for smears, flow cytometry, molecular diagnostic workup, and cytogenetic analysis. Patient recruitment criteria included a full cytogenetic analysis and a routine FISH panel assay workup. Approval for the study was granted by the hospital’s ethics committee.

Cyto genetic investigations
The bone marrow specimens were cultured as a 24-hour unstimulated culture and a 72-hour culture augmented with IL6 (Roche Diagnostics Corp., USA). To improve the mitotic index, 10µl of 10µg/ml colcemid was added to the cultures overnight before harvest the following morning. Standard GTG banding was performed on the metaphase spreads obtained. Where possible, 20 metaphases were fully analyzed for each case. Patients were considered hyperdiploid if they had between 48–74 chromosomes and non-hyperdiploid if they had hypodiploidy, pseudodiploidy, or near tetraploidy.

FISH
A multiple myeloma FISH panel comprising probes for FGFR3/IGH (t(4;14)(p16;q32)), CCND1/IGH (t(11;14)(q13;q32)), IGH/c-MAF (t(14;16)(q32;q23.2)), RB1 (13q14) and TP53 (17p13) (Abbott Molecular, USA) was performed on all samples. A hyperdiploid FISH panel was also available on request, consisting of probes for the centromeres of chromosomes 9, 11 and 15 (Abbott). A second round of FISH was performed with probes for 1q21/1p36 and 6q21/15q22 ( Poseidon, KREATECH Biotechnology B.V, The Netherlands) on the archived bone marrow cell pellets.

FISH assays were performed according to the manufacturers’ specifications. In brief, cell pellets were retrieved for slide-making following bone marrow culture and harvest. Approximately 10 µl of cell suspension was applied to a spot on a slide for each probe application.

Two hundred nuclei were enumerated for each FISH panel probe between two scorers and the average scores were tabulated. Cut-off values were established by the laboratory previously during validation studies. For the IGH dual fusion probes, this was established at 1.48%. The cut-offs for RB1 and TP53 were 5.06% and 10.5%, respectively. One hundred nuclei were enumerated for the 1p36/1q21 and 6q21/15q22 probe sets, between two scorers. The cut-off was set at 8.0%.

RESULTS
Karyotypes were obtained from each of the 45 patients. Among these, 20 patients were considered hyperdiploid, 11 were diploid, and 14 were hypodiploid (Table 1).

The cytogenetic abnormality rate obtained was 75.6% (34/45). The remaining 11 cases had either a normal cytogenetic finding or had only a constitutional abnormality (case 45). Highly complex karyotypes were found among both hyperdiploid and non-hyperdiploid patients. The most frequent numerical gains among hyperdiploid patients were of chromosomes 9, 15, 3, 5 and 19, 8 and 21, and 11, in decreasing order. Among recurring structural aberrations in this group of patients, the most common rearrangements involved deletions around the 1p13 band, followed by deletion of 8q24.1. There were only two cases involving rearrangements of 14q32. Among the non-hyperdiploid patients, monosomy 13 was the most frequent numerical change whereas structural rearrangements of 1p13–p21, 12p11–p13, 14q32, del(1p13), and add(8q24.1) were more common.

The FISH panel results from the same bone marrow samples that were karyotyped showed a close agreement with the chromosomal abnormalities detected except when the lesions were cryptic or where the karyotype abnormalities involved chromosomes/chromosome regions not included in the FISH panels. FISH detected a total of 53 abnormalities of which some were co-existing (Table 2). Of the 23 IGH rearrangements detected, the FGFR3/IGH fusion (18 cases) was the most frequent compared to CCND1 and c-MAF with just 3 and 2 cases, respectively. RB1 deletion/monosomy 13 was most common single abnormality (23 cases, 43.4%) ascertained by the FISH panel. In contrast, there were only 7 cases (13.2%) of TP53 deletion/monosomy 17. The FISH panel increased the detection rate by a further 7 cases so that the combined detection rate was increased from 75.6% to 91.1% (41/45 cases).

With the 1p36/1q21 and 6q21/15q22 probe sets, a total of 47 abnormalities were found. The abnormalities in ascending order were del(1p36) and del(6q21) (4.3% each), amp(1q21) (42.5%), and amp(15q22) (48.9%). The results showed that while amplifications of 1q21 and 15q22, a reflection of trisomy 15, were more frequent events, with just 2 cases each.

DISCUSSION
Although multiple myeloma is the second most common
Table 1. Karyotypes obtained from the 45 newly diagnosed patients

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<tr>
<th>Patients</th>
<th>Karyotype</th>
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<tr>
<td>1</td>
<td>87&lt;4n&gt;,XX,add(X)(p11.2),+1,der(1;16)(q10;10)x2,del(6)(q21q27)x2,+9,del(9p)22p)x2,-12,-13,-14,add(14)(q32)x2,del(21;21)(p11.2;p11.2),+2mar[2]/46,XX[18]</td>
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<td>2</td>
<td>55–57,XX,-X,X,del(1;14)(q10;q10),der(1;pter-&gt;q21;q32-&gt;q13-&gt;qter),+3,+5,+6,+7,+8,+9,+11,der(11)t(1;11)(q12;q25),+add(12) (p11.1),+13,+15,+18,+19,+19,+21,+22,+1-2mar[cp15]/46,XY[5]</td>
</tr>
<tr>
<td>3</td>
<td>84&lt;4n&gt;,XX,Y,YI(q10)x2,+3,+4,-4,-5,add(6)(q12),+7,+8,+inv(9)(p11q13c),+inv(9)(p11q13c),-10,-13,-14,-15,16,46,XY,inv(9) (p11q13c)[16]</td>
</tr>
<tr>
<td>4</td>
<td>53,XY,+3,+5,-11,+15,+19,+19[3]/46,XY[17]</td>
</tr>
<tr>
<td>5</td>
<td>52,XY,+add(1)(p13)x2,+2,add(3)(p21),del(4)(p12p16),der(4;8)(q10;q10),+add(5)(q14),+add(6)(q13),+7,+9,+15,add(17)(p11.2) [68–104&lt;5n&gt;,addemx[2]/46,XY,+1,der(1;10q10;10)1/46,XY[12]</td>
</tr>
<tr>
<td>6</td>
<td>58–59,XY,+1,der(1;16)(q10;p10),-der(1;16),del(2)t(2;6)(q33p11),-der(2)t(2;6),+3,+7,add(8)(q22),+add(11)(q13)x2,+12,der(14)t(11;14) (q13q32)x2,+15,+16,+17,+18,+21,+21[3]/46,XY[22]</td>
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<tr>
<td>7</td>
<td>52–54,XY,add(1)(p13),+3,+5,+10,+11,+19[3]/46,XY[17]</td>
</tr>
<tr>
<td>9</td>
<td>54,X,add(X)(q22),del(1)p13p31),+3,+5,add(6)(q12),+9,+11,+11,der(14)t(14;22)(q32;11.1),+15,+15,+19,+21,22[5]/46,XY[15]</td>
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</table>
| 10       | 47–50,XX,+5,add(6)(p23),+7,add(8)(p12),del(8)(q24;1q24.3),+9,+13,+16,+19,+mar[1](p9;49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,49,
hematological malignancy in the USA, it is relatively rare among Asians. Owing partly to the rarity of the disease, there is a paucity of reports about the chromosomal aberrations in the Southeast Asian population.

Our cytogenetic abnormality detection rate of 75.6% (Table 1) was much higher than the 30%–45% rate reported in the literature. This superior rate could be attributable to the 72-hour culture system with IL6 supplement and the overnight colcemid exposure system we employed to increase the mitotic index in addition to a 24-hour culture. The karyotypes obtained showed numerical and complex structural abnormalities. The chromosomal abnormalities of our hyperdiploid patients appeared to be similar to that in the literature on Western patients with recurrent gains of chromosomes 15, 3, 5 and 19, 8 and 21, and 11, in decreasing order.5,8 Monosomy 13 and structural rearrangements involving t(13p–p21), 12p11–p13, 14q32, and 8q24.1 were more common among our non-hyperdiploid patients. Again, in this respect, the abnormalities were no different to those reported in the literature.

The current study indicated that translocations involving the IGH gene obtained with our IGH probe sets were also very common in our local population with a total abnormality rate of 43.4% (Table 2). These were found far more frequently among non-hyperdiploid patients (data not shown). Of the 23 IGH positive cases, FGFR3 (18 cases, 34%) was the most frequent partner. This particular rearrangement is cytogenetically cryptic because the telomeric bands of chromosomes 4p16 and 14q32 that are reciprocally exchanged are both light bands and of a similar size. Consequently, the t(4;14) rearrangement, and similarly the t(14;16) rearrangement (IGH/MAF), was not detected by our cytogenetics.

In the majority of cases, del(13q) precedes t(4;14) in patients with non-hyperdiploidy.10 Our study found a similar incidence of del(13q), corroborating with other reports that these two abnormalities, IGH translocation and del(13q), are the most frequent aberrations detected by the standard FISH panel. Similar rates have been reported in other Asian countries. In one Japanese study, a combined figure of 28.6% of t(4;14) and t(11;14) abnormalities and a 36% incidence of del(13q) was estimated11 while in a South Korean study, Bang et al12 reported an incidence of 37% of IGH abnormalities and 48% of del(13q). Among a sub-set of the Chinese population, del(13q) was reported to range from 51.4%–63.3%.13 These figures showed that the incidence of del(13q) abnormalities is very high and is quite similar to the West which is reportedly about 50%.10,14-17 However, IGH gene rearrangements in the West have been reported to be even higher with rates of between 55%–75%.18 There is an overlap in the reported rates of IGH rearrangements as some investigators employ an IGHV breakapart probe strategy whereas others reported rates of specific IGH gene fusions. In our study, using an IGHV breakapart probe as the sole assay to determine IGH rearrangements would probably have increased our IGH abnormality rate somewhat closer to the rates reported for Western populations. Indeed, a recent study using the IGHV probe strategy reported a 70% rate of illegitimate IGH rearrangements among Mainland Chinese.10

Of all the prognostic markers, del(17p) is considered as having the worst prognosis because of the loss of the tumor suppressor gene, TP53. Our positive rate of 13.2% was lower than those reported for the Japanese and Mainland Chinese populations, which was more than 20%, according to the reports by Kurashi et al11 and Hu et al,13 respectively.11,13 Only in South Korea and Taiwan, China were the rates reportedly lower, at 5% and 6%, respectively.12,20 These figures were similar to the del(17p) aberration rate in the West of between 10-13% and in the recent report on a Chinese population with a 13.3% abnormality rate.16,17,19,21 The low incidence of del(17p) abnormalities is not unexpected as this abnormality occurs more usually as a late event in the evolution of MM and is more frequently seen at disease relapse.20-24

Abnormalities of chromosome 1 have also been described as a poor prognosticator.15,16,25 Loss of the p-arm of chromosome 1 at bands p13–p21 and gains of the q-arm at q21 (amplification) were reported by Cigudosa et al and Walker et al.26,27 The minimum commonly deleted region in the p-arm was determined to be between 1p12–1p21.1. The use of the KREATech distal 1p36 probe precluded the detection of such deletions. However, our cytogenetics detected deletions in the critical regions of 1p13–p21 in both hyperdiploid and non-hyperdiploid patients. Overexpression of CKS1B at 1q21 has been shown to play an important role in plasma cell proliferation and survival, as well as multi-drug resistance.28 The frequency of amp(1q21) is reportedly about 43% among newly diagnosed MM patients in the USA.5,20 A recent study of 77 newly diagnosed MM patients in China yielded a similar incidence of 45.8%.13 High incidences of amp(1q21) were also reported in other Asian populations, ranging from 45.0%–61.7%.12,19,20 The current study also concurred that amp(1q21) is a common abnormality as a 42.5% incidence was obtained. Therefore, the high prevalence of amp(1q21) in our population was similar to the rest of the world. The amplification of 1q21, at least in our series, is likely to be cryptic in some cases given that the abnormality was not detected by our karyotyping even though it occurred at a very high frequency as detected by FISH.

Gains of chromosome 15 are usually associated with a hyperdiploid karyotype. In this study, 48.9% of the cases

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<th>Abnormal rate</th>
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<tr>
<td>FGFR3/IGH fusion</td>
<td>18 (34%)</td>
<td>del(1p36)</td>
<td>2 (4.3%)</td>
</tr>
<tr>
<td>CCND1/IGH fusion</td>
<td>3 (5.7%)</td>
<td>amp(1q21)</td>
<td>20 (42.5%)</td>
</tr>
<tr>
<td>IGH/MAF fusion</td>
<td>2 (3.7%)</td>
<td>del(6q21)</td>
<td>2 (4.3%)</td>
</tr>
<tr>
<td>del(13q)/monosomy 13</td>
<td>23 (43.4%)</td>
<td>amp(15q22)</td>
<td>23 (48.9%)</td>
</tr>
<tr>
<td>del(17p)/monosomy 17</td>
<td>7 (13.2%)</td>
<td>Total</td>
<td>47</td>
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</table>
had amp(15q22). This was slightly higher than that detected by karyotyping which detected 16 cases of trisomy 15 (36.4%). The disparity might be accounted for by some marker and “add” chromosomes (chromosomes with additional material of unknown origin) that complicated the identification of specific chromosomes. This underscores the importance of including the chromosome 15 enumeration probe for interphase FISH assay for hyperdiploid cases, in addition to probes for chromosomes 5, 9 and 11 which are also commonly seen in hyperdiploid patients.

We have successfully reported the comprehensive cytogenetic profiling of a cohort of newly-diagnosed myeloma patients in our population. This study indicated that the genetic and cytogenetic abnormalities, and their frequencies, associated with multiple myeloma in our sub-set of the Singapore population are generally similar to that reported for the rest of the world. The advent of novel therapy in multiple myeloma and the move towards individualized medicine will imply that successful risk stratification and cytogenetic profiling will be increasingly important in informing therapeutic decisions. In the near future, high through-put chromosome microarray technology may further redefine molecular classification of myeloma. While microarray analysis is still largely unavailable on a routine basis for cancer studies, a comprehensive genetic assessment by conventional karyotyping and interphase FISH will remain the most robust method of cytogenetic profiling for now.

REFERENCES


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