Hyperdiploid Multiple Myeloma with Novel Complex Structural Chromosome Abnormalities Associated with Poor Prognosis: A Rare Case Report

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ABSTRACT

Hyperdiploid multiple myeloma (MM) is associated with better prognosis and non-hyperdiploid subtype is associated with variable to adverse prognosis based on the nature of karyotype abnormality. Rarely exceptions to this hyperdiploid and non-hyperdiploid divisions do exist in a minority. We report an adult male MM patient who showed hyperdiploid karyotype with few novel complex abnormalities and who showed poor clinical outcome. Conventional cytogenetic analysis carried out in 22 GTG banded metaphases showed 53,Y,der(X)t(X;22)(q27;q11.2),+3,+5,+6,+9,+11,+15,der(17)ins(17;1;3)(q11.2;?;?),der(17)ins(17;1;3)(q11.2;?;?),+19,-22,+mar karyotype pattern in 15 metaphases whereas 7 metaphases showed 46,XY karyotype pattern. Interphase FISH revealed biallelic del(13q14) and del(17p13) but no translocations involving the 14q32 region. Through Spectral karyotyping FISH, the origin of complex abnormalities involving der(17) chromosome, translocation t(X;22), and marker chromosome could be clearly delineated. Although the present case showed hyperdiploid karyotype, he showed an adverse prognosis probably due to the co-existence of high risk and complex abnormalities and expired 5 months after initial diagnosis despite standard treatment given.

Keywords: Multiple myeloma; Conventional cytogenetics; Interphase fluorescence in situ hybridization (iFISH); Spectral karyotyping; Novel complex cytogenetic abnormalities

INTRODUCTION

Multiple myeloma (MM) is a clonal B-cell neoplasm characterized by the accumulation of malignant plasma cells in the bone marrow which secrete a monoclonal immunoglobulin (Ig) that is detectable in the serum or urine. According to GLOBOCAN report, 159,985 individuals are estimated to be living with MM globally and comprises approximately 0.9% of all cancers. This terminally differentiated neoplasm of immunoglobulin (Ig) producing plasma cells accounts for approximately 13% of all haematological malignancies. Although
the etiology of MM is unknown, increasing age (>65 years), obesity, race (2 times higher in African Americans), and high body mass index have been implicated as risk factors associated with MM. Although MM is generally an incurable disease, it is highly treatable. Drug resistance and disease refractoriness are the common terminal pathways leading to death. Approval of several new therapies for the treatment of MM has resulted in improved outcomes, but still considerable heterogeneity exists in survival outcomes among MM patients. Some patients experience 10-15 year survivals, whereas others succumb to highly refractory disease within a few months. A key factor underlying the clinical and therapeutic challenge is biological heterogeneity that exists in MM, which is characterized by very complex cytogenetic and molecular genetic alterations. Hence, recognition of clinical or biological parameters predicting the outcome and identifying patients for whom an aggressive therapy could be indicated is essential in MM at diagnosis.

Genetic subtypes of MM have different underlying biologic features and show heterogeneity in clinical outcomes. The identification of high-risk genetic features based on cytogenetics and FISH allows patients to be stratified into the new risk-adapted therapies. Like other hematologic malignancies, there is increasing evidence for the importance of cytogenetic defects, as the most powerful prognostic factor in newly diagnosed MM patients. Cytogenetically, the major division within MM is between hyperdiploid and non-hyperdiploid subtypes. Hyperdiploid myeloma is characterized by trisomies of certain odd numbered chromosomes namely 3, 5, 7, 9, 11, 15, 19 and 21, whereas non-hyperdiploid myeloma is characterized by translocations of the Ig heavy chain alleles at chromosome 14q32 with various partner chromosomes, the most important of which being t(4;14), t(6;14), t(11;14), t(14;16), and t(14;20). Hyperdiploidy is usually associated with a favourable prognosis. Rarely, exceptions to the hyperdiploidy and non-hyperdiploidy divisions do exist in a minority. In this report, we describe an adult male patient diagnosed with MM who presented a hyperdiploid karyotype with usual and unusual trisomies and a set of novel complex abnormalities not previously described and showed adverse prognosis with poor clinical course.

Case presentation
A 69 year-old Malay gentleman was diagnosed to have advanced stage MM when he presented with significant weight loss, abdominal pain, hypercalcemia, dehydration, normochromic normocytic anemia and acute kidney injury. The clinical and biological characteristics of this patient are shown in Table-1. Bone marrow aspiration showed the presence of plasma cells (80%) with abnormal morphology. Radiological investigation showed extensive lytic lesions in his vertebrae, long bones and skull. Serum protein electrophoresis showed increased levels of M protein. Through Durie and Salmon and International Staging System, stage III MM was confirmed.

Table 1: Clinical and biological characteristics of the patient

| Particulars                          | Details                                      |
|--------------------------------------|----------------------------------------------|
| Age                                  | 69 years                                     |
| Sex                                  | Male                                         |
| Stage of disease                     | III                                          |
| Investigations:                      |                                              |
| β 2 microglobulin                    | 9.48 mcg/mL                                  |
| Serum paraprotein                    | 39.1 g/L                                     |
| Ig A kappa monoclonal gammapathy    |                                              |
| Free light chain kappa               | 42.78 mg/L                                   |
| Free light chain lambda              | 475.05 mg/L                                  |
| FLC kappa/lambda ratio               | 0.0901                                       |
| Serum albumin                        | 23 g/L                                       |
| AG reversed                           |                                              |
| Serum calcium                        | 3.98 mmol/L                                  |
| Serum creatinine                     | 270µmo/L                                     |
| Urine protein (24 hours)             | 0.17g                                        |
| Full blood picture                   | Normocytic normochromic anaemia (Hb 8.1 g/dL) with rouleux formation, leucoerythroblastic cells |
| BMA                                  | Presence of plasma cell (80%) with abnormal morphology |
| Skeletal survey                      | Extensive lytic lesions in the vertebrae, long bones and skulls |

Conventional cytogenetic analysis (CCA)
As part of diagnostic workup, bone marrow (BM) aspiration at the time of disease diagnosis was used for conventional cytogenetics and FISH analysis. CCA was carried out using BM from non-stimulated culture in RPMI medium with 20% foetal calf serum after 24 hours. Chromosome preparations were GTG
banded (22 cells) and karyotypes abnormalities were described following ISCN (2016)\textsuperscript{15}. CCA carried out in 22 GTG banded metaphases showed 53,Y, der(X)t(X;22)(q27;q11.2),+3,+5,+6,+11,+15,der(17)ins(17;1;3)(q11.2;?:?),+der(17)ins(17;1;3)(q11.2;?:?),+19,-22,+mar [15] / 46,XY[7] karyotype pattern (Figure 1).

**Interphase fluorescence in situ hybridization (iFISH) analysis**

iFISH analysis was carried out using IGH translocation fusion probes such as t(4;14)(p16.3;q32), t(6;14)(p21;q32), t(11;14)(q13;q32), t(14;16)(q32;q23), t(14;20)(q32;q12) and the deletion probes for del(13)(q14.3) and del(17)(p13), following standard FISH protocol. A minimum of 200 interphase nuclei were screened for all the above abnormalities. FISH analysis showed biallelic deletion of 13q14 and deletion of 17p13 in 64% and 17% of the interphase nuclei, respectively. None of the IGH translocations were observed.

**FISH Spectral Karyotyping (SKY)**

Spectral Karyotyping was carried out using GenASIs Hyper Spectral Karyotyping (HiSKY) Kit containing 24-color combinatorially labeled FISH probes. This allowed visualization of all the human chromosomes at one time by “painting” each pair of chromosomes in a different fluorescence color. SKY analysis was more informative in the identification of a few abnormalities which could not be identified by CCA. SKY identified the abnormal chromosome 17 to be a der(17) resulting from translocation of segment of chromosome 1 and 3 to chromosome 17 and the marker chromosome to be part of chromosome 9 (Figure 2). SKY also clearly delineated the translocation between chromosomes X and 22.
Figure 2. Spectral karyotype image of the patient's metaphase

Treatment
Despite of slight improvement in his renal function following initial treatment with bortezomib-thalidomide-dexamethasone protocol, it has been complicated by recurrent chest infections. This has been going on despite of efforts being made to modify his treatment intensity as well as optimizing supportive care. He succumbed at home about 5 months of diagnosis.

DISCUSSION
Prognosis and risk stratification of MM are based on the revised International Staging system (ISS), Durie–Salmon staging system and genetic classification with chromosomal abnormalities\textsuperscript{16-18}. Chromosome abnormalities account for 40 to 50% of primary events in MM and strongly influence disease phenotype. Based on the findings of CCA and iFISH, the chromosome ploidy status and IGH gene rearrangements are two genetic criteria that are used to help stratify MM patients into prognostic groups. Several cytogenetic abnormalities occur at various time points in the disease course. As reported by Bergsagel et al\textsuperscript{9}, accurate detection and interpretation of cytogenetic abnormalities not only help to discuss available treatment options regarding the anticipated outcomes but also help to make better treatment choices and select the therapeutic strategy. Specific cytogenetic abnormalities affect clinical presentation, progression, prognosis and management strategies of MM\textsuperscript{7,10}. However, the interpretation of cytogenetic results in MM is complicated by the sheer number and complexity of the abnormalities, the methods used to detect them and the disease stage at which they are detected. This patient’s tumor cells exhibited a hyperdiploid chromosome content with 53 chromosomes and included the commonly encountered trisomies associated with good prognosis. Additionally, a set of novel anomalies (+6, der(17), der(X) ) were also present. These abnormalities by themselves and the loss of p53 gene (as demonstrated by iFISH) might have contributed to chromosomal complexity that might have resulted in aggressive disease course. However, among the novel anomalies, the nature of der (17) could not be confirmed using conventional cytogenetics. According to Sawyer\textsuperscript{2}, the low proliferative activity of tumor cell in early stages of the disease and the difficulty in identifying some cryptic translocations are the limitations of CCA
which can be overcome in part by use of iFISH, SKY and Comparative Genomic Hybridization (CGH). So, in the present case, SKY was also employed to identify the cryptic translocations involving chromosome 17. SKY revealed that derivative chromosome 17 included segments from chromosomes 1 and 3, while iFISH revealed deletions of 13q14 and 17p13. The marker chromosome was identified as part of chromosome 9. So, SKY could be an adjunct to conventional karyotyping in quick identification of cryptic translocation and marker chromosomes in genetically complex hyperdiploid MM cases who should be followed up closely because of risk of clinical progression.

Abnormalities involving chromosome 13 are found in about 50% of newly diagnosed MM cases. The most common type of abnormality was monosomy 13 (85%), while the remaining 15% constituted deletion in 13 encompassing the RB1 gene. Recent evidence suggests that the prognostic relevance of del(13) may be related to its association with other genetic alterations. In this patient, IFISH analysis revealed del(13q14) which could be detected only by FISH. Del(13q14) involved both monoallelic and biallelic deletion of 13q14 region. In this patient, biallelic inactivation of RB1 gene was found to be a negative prognostic marker associated with disease progression. Chavan et al. reported that patients with biallelic RB1 inactivation were associated with relapse and poor prognosis. Structural rearrangement of trisomies, especially involving chromosome 17, was a novel finding. Del(17p13) has been detected in higher proportion of relapsed cases. Hence, the presence of del(17p13) detected by FISH is one of the factors associated with worst outcome even in the era of novel therapeutic agents. Because of the submicroscopic nature, del(13q14) and del(17p13) are clearly underscored by CCA, but detected by iFISH in interphase nuclei.

Hyperdiploid and non-hyperdiploid changes appear to represent early or even initiating mutagenic events that are subsequently followed by secondary aberrations including copy number abnormalities, additional translocations, mutations, and epigenetic modifications leading to plasma cell immortalizations and disease progression. Hyperdiploidy involving trisomies of odd numbered chromosomes (3, 5, 7, 9, 11, 15, 17, 19) is an event witnessed in approximately 50% of MM cases, especially in elderly patients. The underlying mechanism which generates hyperdiploidy is unknown, but one mechanism that has been proposed is a single catastrophic mitosis leading to gain of whole chromosomes rather than serial accumulation over time. Associated with high incidence of bone disease, MM patients with hyperdiploid karyotypes have been reported to have a favourable outcome provided that it does not occur together with unfavourable IgH translocations or 17p13 deletions. They also respond particularly well to lenalidomide-based therapy. Although our patient presented with hyperdiploidy and negative unfavourable IgH translocations, he had complex karyotype, biallelic 13q14 and 17p13 deletions, and a poor prognosis. This is in agreement with Carballo-Zarate et al. who reported that the median overall survival of MM patients with hyperdiploid myeloma was negatively correlated with number of additional-structural-chromosomal aberrations. In the present case, it is presumed that trisomies (of chromosomes 3, 5, 6, 11, 15, 17, and 19) occurred early in the pathogenesis of MM, whereas other structural rearrangements such as der(17)ins(17;1;3), der(X)t(X;22) and marker chromosome occurred later.

It is important to note that in newly diagnosed MM patients the abnormal clones have a low proliferative activity and therefore most of the analyzable metaphase cells are derived from normal haematopoiesis. As a result, only about 30-40% of new patients demonstrate an abnormal karyotype by conventional metaphase cytogenetic techniques. According to Rajan and Rajkumar, detection of any cytogenetic abnormality on conventional metaphase cytogenetics indicates a more proliferative form of MM and an adverse prognosis. Thus, the presence of trisomies on metaphase cytogenetic studies does not carry the same good risk implications as they do when detected by FISH. Hence, in the reported case, the detection of chromosomal abnormalities by conventional metaphase cytogenetics itself might have been an indication of adverse prognosis. Furthermore, the detection of complex cytogenetic abnormalities (≥ 3 abnormalities), hypodiploidy,
monosomy 13/del(13q) or monosomy 17/del (17p) on conventional cytogenetics in a patient with MM have also been reported to be indicative of a more adverse prognosis\textsuperscript{11,12}. Complex karyotypic abnormalities were encountered in our patient and that might be yet another adverse sign. Old age of the current patient also might have been another adverse prognostic factor. Worsening outcomes with shorter progression-free survival (PFS) and overall survival (OS) has been reported in MM patients >65 years old in other studies\textsuperscript{24,25}, indicating age also as an important prognostic factor in MM. In a large global study, Ludwig et al\textsuperscript{26} reported that there is a progressive shortening of survival with increasing age even in patients treated with novel agents. These factors seem to be clearly account for the worst prognosis in the present reported case.

CONCLUSION

Complex karyotype and chromosomal deletion of 17p13 are both adverse risk factors in MM. Conventional cytogenetic analysis has its limitations in detecting complex structural chromosomal aberrations due to low chromosomal resolution in bone marrow sample and cryptic nature of some chromosomal rearrangements. Therefore, applying other modality techniques such as SKY and iFISH in detecting complex chromosomal abnormalities are of significant value in classification, risk stratification and management of MM patients. Hyperdiploid MM patients with advancing age, presenting with high risk as well as structurally rearranged complex abnormalities should be followed up closely because of risk of clinical progression. The recent incorporation of high-risk cytogenetic abnormalities into the Revised International Staging System for MM\textsuperscript{10,27} clearly implicates the importance of cytogenetic assessment of MM and its essentiality in clinical practice.

Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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