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Published in:
British Journal of Haematology

DOI:
10.1046/j.1365-2141.2003.04221.x

2003

Link to publication

Citation for published version (APA):

Total number of authors:
8

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A pooled analysis of karyotypic patterns, breakpoints and imbalances in 783 cytogenetically abnormal multiple myelomas reveals frequently involved chromosome segments as well as significant age- and sex-related differences

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Received 15 September 2002; accepted for publication 21 November 2002

Summary. The cytogenetic features (ploidy, complexity, breakpoints, imbalances) were ascertained in 783 abnormal multiple myeloma (MM) cases to identify frequently involved chromosomal regions as well as a possible impact of age/sex. The series included MM patients from the Mitelman Database of Chromosome Aberrations in Cancer and from our own laboratory. Hyperdiploidy was most common, followed by hypodiploidy, pseudodiploidy and tri- /tetraploidy. Most cases were complex, with a median of eight changes per patient. The distribution of modal numbers differed between younger and older patients, but was not related to sex. No sex- or age-related differences regarding the number of anomalies were found. The most frequent genomic breakpoints were 14q32, 11q13, 1q10, 8q24, 1p11, 1q21, 22q11, 1p13, 1q11, 19q13, 1p22, 6q21 and 17p11. Breaks in 1p13, 6q21 and 11q13 were more common in the younger age group. The most frequent imbalances were +9, −13, +15, +19, +11 and −Y. Trisomy 11 and monosomy 16 were more common among men, while −X was more frequent among women. Loss of Y as the sole change and +5 were more common in elderly patients, and −14 was more frequent in the younger age group. The present findings strongly suggest that some karyotypic features of MM are influenced by endogenous and/or exogenous factors.

Keywords: myeloma, chromosomes, karyotype, age, sex.

Cytogenetic investigations of haematological malignancies, particularly acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML) and the myelodysplastic syndromes (MDS), have resulted in a wealth of information of both biological and clinical importance. For example, numerous specific chromosomal abnormalities are now known to correlate closely with several clinical characteristics, such as morphology, immunophenotype and prognosis (Greenberg et al., 1997; Grimwade et al., 1998; Harrison, 2001), and the identification of leukaemia-associated translocations and inversions has paved the way for the detection and characterization of molecular genetic events that are closely associated with the leukaemogenic process (Look, 1997; Rabbits, 2001). Furthermore, the cytogenetic features of ALL, AML and MDS have been shown to vary in relation to age, sex, occupational and iatrogenic exposure, and geographical origin, indicating that the development of chromosomal aberrations is influenced by constitutional as well as exogenous factors (Johansson et al., 1991; Mertens et al., 1993; Pedersen-Bjergaard & Rowley, 1994; Mauritzson et al., 1999, 2002; Albin et al., 2000; Björk et al., 2000, 2001).

Less is known about the karyotypic patterns of multiple myeloma (MM), despite the fact that the incidence of MM is quite similar to that of acute leukaemias (The National Board of Health and Welfare, 2001; Mitelman et al., 2003). The reasons for the relative lack of published cytogenetically abnormal MM patients include low mitotic index and poor chromosome morphology (Nilsson et al., 2002). In addition, the cytogenetic complexity of MM is, at least compared with ALL, AML and MDS, quite pronounced. which, in the past,
has hampered the identification of biologically and clinically important chromosomal abnormalities. However, during the last few years, some cytogenetic features have been shown to be associated with certain clinical parameters in MM. For example, loss of chromosome 13 material and hypodiploidy seem to confer a worsened prognosis (Tricot et al., 1995; Desikan et al., 2000; Smadja et al., 2001), and t(11;14)(q13;q32)-positive MM patients are characterized by a lymphoplasmacytic morphology (Fonseca et al., 1998; Hoyer et al., 2000). Furthermore, molecular genetic investigations have clearly shown that illegitimate recombinations of the immunoglobulin heavy chain (IGH) gene, at chromosome band 14q32, occur in the vast majority of MM, resulting in dysregulation of the target genes (Kuehl & Bergsagel, 2002). However, very little is known about the molecular genetic consequences of other chromosomal anomalies in MM. To some extent, this can be explained by the lack of a detailed and genome-wide cytogenetic map of MM that could form the basis for further molecular genetic analyses.

In the present study, karyotypically abnormal MM, including patients from the Mitelman Database of Chromosome Aberrations in Cancer (Mitelman et al., 1995) and from our own laboratory, were collected and reviewed cytogenetically. The aim was to construct genomic breakpoint and imbalance maps, delineating regions likely to harbour genes of pathogenetic importance. In addition, a pooled statistical analysis evaluating the possible impact of age and sex on the karyotypic features was performed.

MATERIALS AND METHODS

Patients. The study was based on all cytogenetically abnormal MM analysed in our laboratory between 1978 and 2000; the vast majority were obtained after 1995. Clinical and karyotypic data on all but a few of these patients have been reported previously (Nilsson et al., 2002). In addition, the Mitelman Database of Chromosome Aberrations in Cancer (Mitelman et al., 2003) and from our own laboratory, were collected and reviewed cytogenetically. The aim was to construct genomic breakpoint and imbalance maps, delineating regions likely to harbour genes of pathogenetic importance. In addition, a pooled statistical analysis evaluating the possible impact of age and sex on the karyotypic features was performed.

Cytogenetic classification. All patients were subgrouped in relation to ploidy levels, in accordance with the International System for Human Cytogenetic Nomenclature (ISCN, 1995), as follows: hypodiploidy (35–45 chromosomes), pseudodiploidy (46 chromosomes), hyperdiploidy (47–57 chromosomes) and tri-/tetraploidy (58–103 chromosomes). Patients containing clones of different ploidy levels were grouped according to the lowest modal number. The MM patients were also subdivided according to the number of abnormalities, i.e. one or two abnormalities or complex karyotypes (more than two aberrations). Patients with clones harbouring different numbers of aberrations were grouped in relation to the most simple clone present, and patients who were incompletely karyotyped, i.e. containing ‘inc’ in the karyotype, were included in the complex group. In the cytogenetic classification and in the breakpoint and imbalance maps (see below), constitutional chromosomal abnormalities were disregarded.

Genomic breakpoint map. For the breakpoint (bp) map, if more than one copy of the same chromosome aberration was found in the same or related clone, the bp involved was plotted only once and, if the same bp was involved in different aberrations, it was plotted once per aberration. Furthermore, rearrangements with an uncertain bp localization, e.g. add(14)(q32) or del(6)(q13–16), were not included.

Genomic imbalance map. The imbalances were ascertained according to the following criteria: (1) the net results of the aberrations were always registered with regard to the nearest ploidy level; (2) when additional chromosomal changes were acquired during clonal evolution, only novel abnormalities were included, i.e. if the same imbalance was found in more than one related clone, it was recorded only once; (3) when the same chromosome was involved in several aberrations, only the total net imbalances were plotted; and (4) in cases in which a particular chromosome segment was involved in different types of imbalances, only the largest imbalance was registered. Clones with loss of chromosome Y as the sole anomaly were not included in the imbalance map.

Statistical investigations. For comparing independent groups of observation by significance testing, i.e. cytogenetic features in relation to age and sex, we used the chi-square test. Two age groups were defined for the analyses: ≤61 years and ≥62 years. The P-values reported are two-sided and P < 0.05 was considered to be significant. In an attempt to focus on pathogenetically important changes, only genomic breakpoints and imbalances found in more than 5% and 10%, respectively, of the MM patients were included in the statistical analyses.

RESULTS

A total of 783 cytogenetically abnormal MM cases were retrieved, including 40 patients from our own laboratory and 743 patients from the database. The series comprised 442 men and 341 women, with a median age of 61.5 years (information on age was lacking in 334 patients). The most common modal number was hyperdiploidy (308/783: 39%), followed by hypodiploidy (212/783: 27%), pseudodiploidy (188/783: 24%) and tri-/tetraploidy (75/783: 10%) (Table I). Most patients (567/783: 72%) were karyotypically complex, whereas one or two abnormalities were found in 170 (22%) and 46 (6%) MM patients respectively. The median number of chromosomal abnormalities per MM patient was eight (Fig 1). More than one cytogenetically abnormal clone was identified in 71 patients (9%), of whom 19 (27%) had unrelated clones and 52 (73%) displayed a clonal evolution.

Among the 170 MM patients with sole chromosomal aberrations, 55 (32%) had numerical anomalies, most frequently loss of the Y chromosome (26 patients), and monosomies X, 7, 18 and 21 (three patients each), while 115 (68%) displayed structural rearrangements, with t(11;14)(q13;q32) (12 patients), add(14)(q32) (seven
patients, del(1)(p11p22) (four patients), and add(2)(p25); del(11)(q13), del(16)(q22), del(17)(p11) and add(17)(p?) (three patients each) being most common.

The cytogenetic features, i.e. ploidy levels, number of anomalies, and the most frequent genomic breakpoints and imbalances, of the 783 MM patients in relation to sex and age are given in Table I. The modal numbers did not vary in relation to sex, whereas their distribution differed between the two age groups (P = 0.02) with, in particular, hyperdiploidy being more common in elderly patients. No sex- or age-related differences as regards number of anomalies were discerned.
A total of 3176 genomic breakpoints were mapped in the 783 MM patients (Fig 2). The most frequently affected chromosome bands were 14q32 (29%), 11q13 (16%), 1q10 (14%), 8q24 (10%), 1p11 (8%), 1q21 (7%), 22q11 (7%), 1p13 (6%), 1q11 (6%), 19q13 (6%), 1p22 (5%), 6q21 (5%) and 17p11 (5%). There were no frequency differences between men and women, but breaks in 1p13, 6q21 and 11q13 were more common in younger patients ($P < 0.02$, $P < 0.02$ and $P < 0.002$ respectively; Table I).

The genomic imbalances identified are depicted in Fig 3, the most common of which were $+9$ (27%), $-13$ (27%), $+15$ (24%), $+19$ (22%), $+11$ (20%) and $-Y$ (20% of 442 men). Both sex- and age-related differences were noted (Table I). Trisomy 11 and monosomy 16 were more common among men ($P < 0.001$ and $P = 0.02$ respectively), $-X$ was more frequent among women ($P < 0.001$), $-Y$ as the sole change and $+5$ were more common in elderly patients ($P < 0.001$ and $P = 0.008$ respectively), and $-14$ was more frequent in the younger age group ($P = 0.02$).

DISCUSSION

Because of the heterogeneous nature of the ascertained MM patients, including samples analysed in various laboratories during different time periods and by different chromosomal banding techniques, karyotypic errors undoubtedly exist in the reviewed material. In order to minimize the effects of these abnormalities with questionable breakpoint mapping were excluded. Furthermore, only breakpoints and imbalances reported in more than 5% and 10% of the patients, respectively, were included in the statistical analyses. By thus decreasing the impact of cytogenetic uncertainties/mistakes and karyotypic noise, we believe that the abnormalities and abnormality patterns delineated in the present study (Figs 1–3, Table I) should be representative for MM.

The most common modal number was hyperdiploidy, followed by hypodiploidy, pseudodiploidy and tri-/tetraploidy (Table I). This distribution of ploidy levels differed from those seen in monoclonal gammopathy of undetermined significance (MGUS) and in plasma cell leukaemias (PCL) – the vast majority of MGUS with cytogenetic abnormalities reported to date have been pseudodiploid and almost 50% of PCL are hypodiploid, with the remaining being pseudodiploid or hyperdiploid in equal frequencies – but is quite similar to the one observed in Waldenström’s macroglobulinaemia (WM), which is hyperdiploid, pseudodiploid or hypodiploid in approximately one third of the patients respectively (Palka et al., 1987; Calasanz et al., 1997; Avet-Loiseau et al., 2001; Mitelman et al., 2003). Also the cytogenetic complexity seems to vary among the different plasma cell dyscrasias. The vast majority of PCL and MM are karyotypically complex, with half of the MM patients harbouring more than eight chromosomal aberrations (Fig 1), whereas most MGUS and WM patients have only one or two abnormalities (Mitelman et al., 2003). Compared with other haematological malignancies, the karyotypic pattern of MM is similar to those found in follicular lymphomas and diffuse large B-cell lymphomas, but quite different from the ones seen in, for example, B-cell chronic lymphocytic leukaemia and myeloid malignancies, such as AML and MDS (Mauritzson et al., 2002; Mitelman et al., 2003). Taken together, the basic cytogenetic features, i.e. ploidy levels and degree of complexity, of MM compare well with those characterizing PCL, WM and lymphomas.

Among the 3176 genomic breakpoints in MM ascertained herein, more than a third of them clustered to 1p22, 1p13, 1p11, 1q10, 1q11, 1q21, 6q21, 8q24, 11q13, 1q32, 17p11, 19q13 and 22q11 (Table I and Fig 2). It should be stressed that, in this context, the vast majority of the reviewed karyotypes were based on G-banding alone.
and that cryptic translocations, identifiable only by fluorescence in situ hybridization (FISH) or pure molecular genetic techniques, generally were not included. Hence, in the present breakpoint map (Fig 2), there is an under-representation of breaks in, e.g. 4p16 and 16q23, known to be affected by the common MM-associated t(4;14)(p16q32) and t(14;16)(q32;q23) (Kuehl & Bergsagel, 2002). Furthermore, although 3176 breakpoints is an impressive figure, it is probably an underestimation, considering that recent multicolour spectral karyotyping (SKY) analyses of MM have identified several novel recurring sites of breakage that were not identifiable by conventional chromosome banding (Rao et al, 1998; Sawyer et al, 1998a, 2001; Ng et al, 2001). Despite this, the frequently involved chromosome bands identified in this study (Fig 2) probably harbour genes of importance in the development and/or progression of MM. However, apart from the known involvement of the MYC, CCND1, IGH and IGL genes, as a result of breaks in 8q24, 11q13, 14q32 and 22q11, respectively (Kuehl & Bergsagel, 2002), the molecular genetic consequences of these rearrangements remain to be elucidated. Although structural aberrations of genes located in the breakpoint regions are a probable result, one cannot exclude the possibility that genomic imbalances are the important outcome. For example, breaks, such as whole-arm translocations and jumping translocations (Keung et al, 1998; Sawyer et al, 1998b), in the pericentromeric region of chromosome 1 (Fig 2) have been suggested to lead to gene dosage effects rather than to structural aberrations of single genes (Le Baccon et al, 2001). In fact, most of the observed breakpoints were involved in unbalanced rearrangements, resulting in loss or gain of genetic material.

The most frequent, i.e. >10%, genomic imbalances in MM were trisomies and monosomies, in particular +9, +11, −13, +15, +19 and −Y, losses of 1p and 6q, and gain of 1q (Table I). Although some, or even several, abnormalities may have escaped detection by conventional chromosome banding analyses – a large proportion (roughly 20%) of MM patients had multiple marker chromosomes – a high incidence of the above-mentioned gains and losses has also been detected by comparative genomic hybridization analyses (Avet-Loiseau et al, 1997; Cigudosa et al, 1998; Gutiérrez et al, 2001). Thus, the present genomic imbalances of MM (Fig 3) should be valid. However, SKY analyses have revealed that some of the imbalances, mainly those leading to partial losses, are generated through a different mechanism than suggested by G-banding alone. For example, many abnormalities identified as deletions are, in fact, derivative chromosomes of unbalanced translocations (Rao et al, 1998; Sawyer et al, 1998a, 2001). Because partial gains or losses of chromosomes that often are trisomic or monosomic are notably rare (Fig 3), with the exception of dup(11q) and del(13q), the pathogenetically important effect of these numerical changes is probably not reducible to minimally duplicated or deleted segments, and hence not to the altered expression of only a few genes. Considering that a recent microarray-based gene expression analysis of AML patients with trisomy 8 as a sole anomaly showed that numerous genes on this chromosome were overexpressed (Virtanen et al, 2001), it is also reasonable to suggest a similar gene–dosage effect of the trisomies in MM. However, identification of the relevant genes will be an arduous task, although recently reported (Shaughnessy et al, 2001; Claudio et al, 2002; Zhan et al, 2002) and ongoing microarray analyses of MM may prove fruitful in this respect.

Significant age- and sex-related differences in karyotypic patterns have previously been identified in several haematological malignancies. Well-known examples of the impact of age include: the higher frequencies of t(4;11) in infant ALL; t(8;21), t(15;17) and inv(16) in younger AML age groups; and deletions involving chromosomes 5 and 7 in elderly AML patients (Johansson et al, 1998; Mauritzson et al, 1999; Moorman et al, 2001). The influence of sex is generally less pronounced (Mertens et al, 1993). However, there is a preponderance of women with the '5q- syndrome’ and with chronic myeloproliferative syndromes with trisomy 8 as the sole change, whereas the der(1;7)(q10;p10) in myeloid malignancies, in particular MDS, is more common in men (Pedersen, 1992; Boulwood et al, 1994; Paulsson et al, 2001; Mitelman et al, 2003). In MM, on the other hand, most studies addressing this issue have only compared the frequencies of normal and abnormal karyotypes, without detecting any significant age- or sex-related differences (Gould et al, 1988; Weh et al, 1993; Cigudosa et al, 1994; Calasanz et al, 1997; Nilsson et al, 2002). Only a few investigations have focused on specific chromosomal aberrations. Malgeri et al (2000) found no significant correlations between the presence of t(4;14)(p16q32) and age and sex, whereas t(8;22)(q24;q11) is more common among men (Yamamoto et al, 1998; Mitelman et al, 2003). The available data are somewhat conflicting with regard to the t(11;14)(q13;q32). There were no apparent age- or sex-related differences among the t(11;14)-positive MM patients reviewed by Laï et al (1998), whereas Fonseca et al (2002a) reported that patients below the age of 40 years appeared (P = 0.08) to be more likely to have t(11;14). In the present study (Table I), the frequencies of t(11;14) did not differ between men and women, but seemed to be higher in the younger age group (16% vs 10%, P = 0.07). Although this association was not significant, a skewed age distribution of MM patients with all types of abnormalities involving 11q13 was found, with 11q13 rearrangements clearly being less common (P = 0.002) in elderly patients (Table I). Studies of −13/13q− have also yielded contradictory results. Zojer et al (2000) and the present study found no significant associations with age or sex (Table I), whereas Facon et al (2001) and Fonseca et al (2002b) reported higher frequencies of such aberrations in women. Facon et al (2001) also detected a significant association with age: patients with losses of chromosome 13 material were significantly older. Finally, our pooled analysis revealed additional changes displaying age- or sex-related frequency heterogeneity (Table I). For example, +5 was more common in the older...
age group and +11 was more frequent in men. The relatively high frequency of –Y, also as a sole change, among elderly men (Table I) most likely reflects the well known age-related loss of this sex chromosome (Stone & Sandberg, 1995; Herens et al., 1999). As regards chromosome X, only three MM patients with –X as a sole anomaly have been reported (Mitelman et al., 2003), precluding meaningful statistical analyses of the impact of age.

Finally, although some of the observed frequency differences may be fortuitous, the present findings strongly suggest that some karyotypic features of MM are influenced by endogenous and/or exogenous factors.

ACKNOWLEDGMENTS

This work was supported by grants from the Swedish Cancer Society, the John and Augusta Persson Foundation for Medical Research, and Lund University Medical Faculty.

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myeloma is associated only with partial or complete deletions of chromosome 13 or abnormalities involving 11q and not with other karyotype abnormalities. *Blood*, **86**, 4250–4256.


