Title:

Gender Disparities in the Tumor Genetics and Clinical Outcome of Multiple Myeloma

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Abstract

Background:

Several cancer types have differences in incidence and clinical outcome dependent on gender, but these are not well described in myeloma. The aim of this study was to characterize gender disparities in myeloma.

Methods:

We investigated the association of gender with the prevalence of tumor genetic lesions and the clinical outcome of 1960 patients enrolled in the phase III clinical trial MRC Myeloma IX. Genetic lesions were characterized by FISH.

Results:

Disparities were found in the prevalence of primary genetic lesions with *IGH* translocations being more common in women (50% of female patients vs 38% of male patients, $p<0.001$) and hyperdiploidy being more common in men (50% female vs 62% male, $p<0.001$). There were also differences in secondary genetic events with del(13q) (52% female vs 41% male, $p<0.001$) and +1q (43% female vs 36% male, $p=0.042$) being found more frequently in female myeloma patients. Female gender was associated with inferior overall survival (median 44.8 months female vs 49.9 months male, $p=0.020$).

Conclusions:

We found gender-dependent differences in the prevalence of the primary genetic events of myeloma, with *IGH* translocations being more common in women and hyperdiploidy more common in men. This genetic background may impact subsequent genetic events such as +1q and del(13q), which were both more frequent in women. The higher prevalence of lesions associated with poor prognosis

in the female myeloma population, such as t(4;14), t(14;16) and +1q, may adversely affect clinical outcome.

Impact:

These differences suggest that gender influences the primary genetic events of myeloma.

Introduction

Several cancer types have differences in incidence and clinical outcome dependent on gender (1). Lung cancer, for example, is more common in men, and women with lung cancer have better survival than men(2). Moreover, a sex-specific tumor genomic profile has been described in lung cancer, strongly suggesting that there is a gender-specific phenotype (2-3). These data suggest that gender can influence the etiology and natural history of some malignancies.

In myeloma the primary genetic lesions that give rise to a clonal plasma cell population are hyperdiploidy and immunoglobulin heavy chain gene (*IGH*) translocations (4). Hyperdiploidy in myeloma is characterized by gain of multiple odd numbered chromosomes, and the events giving rise to this abnormality are not well understood. *IGH* translocations arise following aberrant class switch recombination events during B cell differentiation, and feature reciprocal translocation of the *IGH* allele at 14q32, usually with one of 5 partner oncogenes (*MMSET, CCND1, CCND3, MAF* or *MAFB*) (5), (6). These two etiological pathways have been used to classify myeloma patients into a hyperdiploid group, and non-hyperdiploid group characterized by a high rate of *IGH* translocations (5, 7). These early genetic events give rise to a clonal plasma cell population, with further events such as structural chromosomal abnormalities, mutation and epigenetic changes required for progression to malignancy (8-10).

It is unknown why these pathogenic events occur in certain individuals, but evidence of genetic susceptibility is suggested by an increased incidence of myeloma in first degree relatives of myeloma patients, within some family groups, and in certain racial groups. Several large population-based studies have found that first-degree relatives of subjects with myeloma or monoclonal gammopathy of undetermined significance

(MGUS) have an increased risk of developing a plasma cell dyscrasia (11-13). Further evidence of hereditable susceptibility is provided by familial clusters of MGUS and myeloma (14-16). Racial background has been shown to be important, with myeloma being twice as common in African Americans as white Americans and least common in Americans of Asian origin(17). The pre-malignant condition that precedes myeloma, MGUS, has a similar pattern suggesting that the higher rate of myeloma in African Americans is due to different rates of primary genetic events as opposed to secondary progression events (18-19).

Gender could also exert similar effects. We have investigated the relationship of gender to the risk of developing myeloma, the prevalence of tumor genetic lesions and the clinical outcome of patients enrolled in the MRC Myeloma IX trial.

Method

Patients

1960 patients were enrolled in the MRC Myeloma IX phase III clinical trial (ISRCTN68454111) (MREC/02/8/95), the design and results of which are described elsewhere (20). Patients over the age of 18 years newly diagnosed with symptomatic myeloma requiring treatment were eligible for selection. Exclusion criteria were concurrent active malignancy excluding basal cell carcinoma and other *in situ* carcinomas, previous myeloma therapy and acute renal failure not responsive to rehydration. The trial compared conventional induction chemotherapy with a thalidomide-based regimen, and incorporated high dose melphalan for younger, fitter patients. Median follow-up was 3.7 years.

FISH

Diagnostic bone marrow aspirates were purified for plasma cells using CD138 magnetic microbeads (Miltenyi Biotec). Material for fluoresence in situ hybridization (FISH) analysis was available from 58.2% of the enrolled patients (1140 patients). Probes were chosen to detect the presence of an *IGH* translocation, the common *IGH* translocation partners (4p16, 6p21, 11q13, 16q23 and 20q12), hyperdiploid status using the iFISH ploidy classification, deletion of 1p32, 13q14, 16q23, 22q11 and gain of 1q21 as previously described (21-22).

Statistical Methods

Statistical analysis tools were SPSS v.19 and R. Analysis of differences in baseline clinical and laboratory variables used the Fisher exact, χ^2 and the nonparametric Wilcoxon tests. Progression free survival (PFS) and Overall Survival (OS) were calculated from Kaplan-Meier curves, with the difference between the curves analyzed using the log-rank test. Multivariate analysis was performed using the proportional hazards regression model of Cox. All p values were two-sided, and values <0.05 were taken as significant.

Results / Discussion

Of the 1960 patients enrolled in the trial, 1165 (59.4%) were male and 795 (40.6%) were female. These figures are consistent with population-based statistics, with incidence rates of 4.4 per 100,000 in men and 2.9 per 100,000 in women equating to a 60/40 split (17). There were few significant differences in baseline clinical and laboratory variables (supplementary data Table 1). The median age of female trial patients was two years older than for men (64 years male vs 66 years female, p=0.007) and female patients were associated with higher levels of serum lactate dehydrogenase, which have been linked to adverse prognosis (median 320 U/L male vs 345 U/L female, p<0.001) (23).

The different incidence of myeloma in men and women suggests that gender may influence etiological events. In this context we found differences in the rates of primary pathogenetic lesions dependant on gender (Table 1). *IGH* translocations were more common in women (50.1% of female patients vs 37.9% of male patients, p<0.001). When the *IGH* translocations were examined based on the 5 common partner genes, all groups were found at higher frequencies in female patients, with the most significant differences seen in the t(4;14) group (14.7% of female patients vs 9.3% of male patients, p=0.009) and t(14;16) group (5.7% of female patients vs 1.6% of male, p<0.001). Conversely, hyperdiploidy was more common in men than women (49.7% of female patients vs 61.7% of male patients, p<0.001).

A range of chromosomal regional deletions and gains were examined and both del(13q) and +1q were found to be more frequent in female patients (del(13q): 52.3% of female patients vs 40.6% of male patients, p<0.001; +1q: 43.1% of female patients vs 36.2% of male patients, p=0.042). Both these lesions showed a significant positive association with *IGH* translocations in the overall dataset, and were negatively associated with hyperdiploidy so it is likely that their increased frequency in female patients was a secondary consequence of the underlying rates of hyperdiploidy and *IGH* translocations. No gender differences were seen in the prevalence of del(1p), del(16q), del(17p) or del(22q), and there were no differences in the percentage of men and women with abnormal karyotypes by conventional cytogenetics (Table 1).

Survival differences were observed when comparing the sexes, with female gender being associated with impaired OS (median OS 44.8 months female vs 49.9 months male, p=0.020) (figure 1) (supplementary data Table 2). There was also a trend towards impaired PFS for women which did not reach a level of significance (median 16.0 months female vs 19.9 months male, p=0.105). The association of gender with

OS was not significant in multivariate analysis, suggesting that competing variables were involved (supplementary data Table 3). Age may play a small role, as the median age of women in the trial was 2 years older than men, and when age-adjusted the association of female gender with impaired survival becomes less significant (p=0.079). However, more significant is the fact that the genetic lesions that were more common in women (t(4;14), t(14;16), del(13q) and +1q) were all strongly associated with impaired survival in univariate analysis, with t(4;14), t(14;16) and +1q being associated with short survival in multivariate testing (supplementary data table 3). In bivariate analysis with any of these lesions, gender ceases to be significantly associated with OS, so the genetic background of male and female patients is likely to play an important role in the observed survival differences. Overall, we think that the moderate impairment in survival associated with female gender mainly reflects the increased prevalence of adverse genetic lesions in female myeloma patients.

A caveat of any clinical trial study, when compared to a population-based study, is that trial selection criteria may introduce bias that affect the generalizability of findings to the overall population. In comparison with many clinical trials, the inclusion and exclusion criteria of the MRC Myeloma IX trial were not strict so that trial participants were representative of the general myeloma population. Unusually, it was a trial designed for myeloma patients of all ages, with the age range of enrolled patients being 31 – 89 years, with a median age of 65. This is slightly lower than the median age at diagnosis reported in population statistics (69 years), perhaps reflecting a reluctance of clinicians to enter older, frailer patients into clinical trials (17). To address this possible bias we specifically examined the prevalence of genetic abnormalities in patients aged 70 or over (n=380). This subgroup analysis corroborated the original findings, with *IGH* translocations being more common in older female patients (48.6% of female patients vs 34.6% of male patients, p=0.013)

and hyperdiploidy being more frequent in older male patients (50.4% of female patients vs 65.6% of male patients, p=0.008). The gender mix of the trial patients was identical to population data, suggesting that gender selection was unbiased. 97% of patients were Caucasian, so these findings require validation in other ethnic groups.

In summary we found gender-dependent differences in the prevalence of *IGH* translocations and hyperdiploidy in newly presenting myeloma patients, with *IGH* translocations being more common in women and hyperdiploidy more common in men. This genetic background may impact subsequent genetic events such as +1q and del(13q), which were both more frequent in women. The relevance of these findings is that it helps to explain the observed gender-dependant survival differences, with female gender being associated with impaired survival due to the increased frequency of genetic lesions associated with poor clinical outcome, especially t(4;14), t(14;16) and +1q. Moreover, it also implies that gender may influence the etiological events of myeloma. Women have a lower risk of developing myeloma, and are more likely to develop myeloma as a result of aberrant class switch recombination events. Conversely, men have a higher risk of developing myeloma, and are more likely to develop myeloma as a result of hyperdiploidy. Whilst a genetic basis for myeloma risk has been suggested to be due to variation in genes associated with innate immunity or cell cycle, these have not been reported to be different in men and women (24-25). It is possible that *IGH* translocations or hyperdiploidy may in some way be influenced by variation in genes situated on the sex chromosomes, or by hormonal differences between men and women, and this should be a focus for further study.

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Table Legend

Table 1. A comparison of the incidence of tumor genetic lesions detected by FISH in male and female patients.

Figure Legend

Figure 1. Overall survival of male and female patients in the Myeloma IX trial. Female median OS was 44.8 months compared with 49.9 months for males.