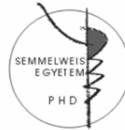


The effect of various genetic and clinical factors on multiple myeloma

PhD Thesis

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Introduction

Multiple myeloma (MM) is a malignant hematological disease caused by the proliferation of transformed plasma cells in the bone marrow. According to the WHO classification it is a peripheral B-cell neoplasm. There are probably around 350–400 new cases per year in Hungary. As a result of the new treatment options in the last decade the survival improved a lot, now in the good prognostic group with stem cell transplantation it is expected to be close to ten years.

The most common presenting symptoms include bone pain, bone marrow failure and in 10% of the cases renal insufficiency. The last decade witnessed the development of several new treatment strategies which have revolutionized the therapy of MM gradually replacing the chemotherapy based approach. This resulted in a decrease in toxicity along with an improvement in effectivity. In younger and fitter patients autologous stem cell transplant is part of the standard first line treatment

Interleukin 6 (IL6) has great importance in myeloma, it enhances the proliferation and survival of MM cells, its blood level reflects the activity of the disease. It is encoded by the IL6 gene. The IL6 promoter -174G>C polymorphism (rs1800795) is a single nucleotide polymorphism (SNP). In carriers of the C allele the lipopolysaccharide (LPS) related IL6 expression is absent.

The interleukin-6 receptor (IL6R) Asp358Ala (rs2228145) is also an SNP, here, in carriers of the C allele (Ala) there is an increase in the soluble form of IL6R. The mechanism of this is not 100% clarified, the most probable explanation is the increased proteolytic shedding of IL6R. This causes increased IL6 transsignalisation with a possible effect on the quality of the inflammatory response.

Nuclear factor kappa B (NFkB) is also very important in MM and indeed in most B-cell malignancies. Its activation increases the proliferation of cells and blocks apoptosis. It was profoundly studied in MM, as bortezomib, one of the most important drug in myeloma blocks this pathway.

The NFkB1 -94ins/delATTG (rs28362491) is an insertion/deletion polymorphism of four bases in the promoter region of the NFkB1 gene. The del allele is less active, the transcription factors have a less

pronounced effect on it compared to the wild type ins allele. For example following LPS stimulation the NFKB1 response is weaker in carriers of the del allele.

We hypothesized that the more active alleles could increase the susceptibility to MM and/or affect the effectivity of treatment.

Aims

1. Given how important the IL6 system is in MM and how much the IL6 promoter -174G>C and the IL6R Asp358Ala polymorphisms affect its responsiveness we considered examining their connection with the prevalence of MM.
2. NFKB is also centrally important in MM. Given that the NFKB1 -94ins/delATTG is relevant in many types of cancer we planned to investigate its role in myeloma.
3. We also wanted to study whether the NFKB1 variants can alter the effectivity of MM treatment.
4. This effect could be different in various subgroups; we also planned to look into this.
5. As bortezomib acts via blocking NFKB1 the survival of the subgroup of bortezomib treated patients was particularly important.
6. All doctors treating MM experienced how long patients sometimes wait until finally get a diagnosis. As there has not yet been a systematic survey of this question in Hungary, we took the opportunity to collect data regarding the presentation symptoms and delays to investigate a possible connection with treatment outcome.

Methods

In the IL6 study 100 MM patients participated who were treated at the Semmelweis University 3rd Department of Internal Medicine between February 2000 and December 2004. As control we used an age adjusted cohort of inpatients with no hematological, oncological or immunological disease.

In the NFKB1 study 295, patients participated who were newly diagnosed and had first line treatment at the Szt. István and Szt.

László Hospital between January 2004 and September 2013. Here the control consisted of 149 blood donors.

In the diagnostic delay analysis the study group was based on the previous cohort, however we only used patients if the information regarding the presenting symptoms was available (n=193).

The IL6R Asp358Ala SNP was analyzed with fluorogenic 5' nuclease (TaqMan) technique at the Department of Genetics, Cell and Immunobiology Institute, Semmelweis University. The IL6 -174G>C SNP was analyzed using restriction fragment length polymorphism (RFLP) technique at the Research Laboratory of the Semmelweis University 3rd Department of Internal Medicine. The NFKB1 -94ins/delATTG polymorphism was also tested using RFLP at the Hungarian National Blood Transfusion Service Laboratory of Molecular Diagnostics.

Results

Population genetic studies

The two SNPs affecting the IL6 system were tested in 92 and 97% of the 100 individual with MM. We did not find any significant differences neither in allele frequencies (AF±95%CI), nor in the distribution of the three genotypes compared to the controls. In case of the IL6R Asp358Ala 34 (37%) patients had homozygous AA, 46 (50%) heterozygous AC and 12 (13%) CC genotype. Regarding the IL6 -174G>C 37 (38.1%) had GG, 43 (44.3%) GC and 17 (17.5%) CC genotype (*Figure 1*).

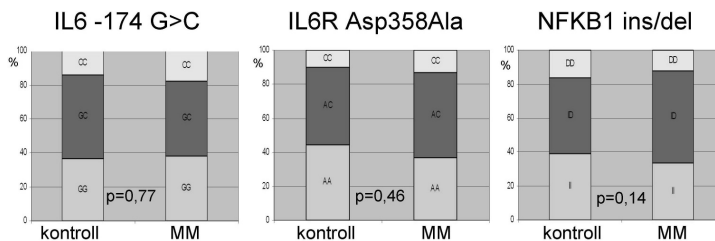


Figure 1. The distribution of the genotype variants of the three analyzed polymorphisms in MM patients and controls

The NFKB1 -94ins/delATTG was analyzed in 295 patients. We found homozygous ins/ins genotype in 99 (33.6%), heterozygous in 35 (11.9%) patients. There were no significant differences neither in allele frequencies nor in the distribution of the genotypes compared to the control. Due to the low case numbers the del/del group was merged into the ins/del.

NFKB1 variants and response to treatment

The distributions of best post therapy responses achieved were the same in the three NFKB1 -94ins/delATTG subgroups: the percentage of CRs, VGPRs, PRs and NR/PDs were 37.5%, 16.7%, 36.5%, 9.4% among the ins/ins patients, and 39.9%, 18.1%, 28.2%, 13.8% among the ins/del&del/del group respectively without significant differences.

However when we analyzed the effect of NFKB1 -94ins/delATTG genotype on survival we found that patients with at least one del allele had a significantly reduced progression free survival (PFS). The median PFS was 670 days in the whole cohort, 790 days in the ins/ins group and 624 days in the ins/del&del/del group ($p = 0.013$). Regarding the overall survival (OS) we found no significant differences (*Figure 2*).

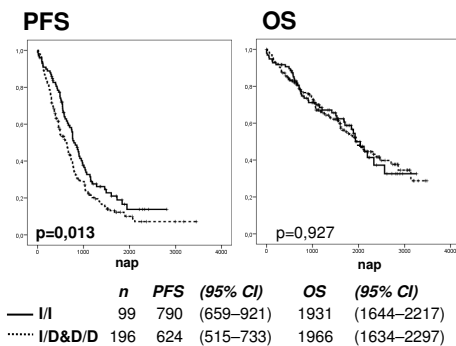


Figure 2. PFS and OS in the two NFKB1 genotype groups

In multivariate analysis, together with age, ISS, FISH and bortezomib exposure, the NFKB1 ins/del&del/del genotype was an independent significant risk factor for shorter PFS.

To summarize the results of the subgroup analyses we found a significant benefit in ins/ins patients all the good prognostic subgroups. The PFS of ins/ins patients was significantly longer in the ISS I-II, FISH good risk groups and also in patients treated with bortezomib containing regimens, while in the ISS III, FISH high risk and non bortezomib patients had a poor outcome regardless their genotype. Low risk ISS and bortezomib treatment were associated with a good outcome in ins/ins patients while in the del allele carriers the prognosis was poor regardless ISS and bortezomib exposition.

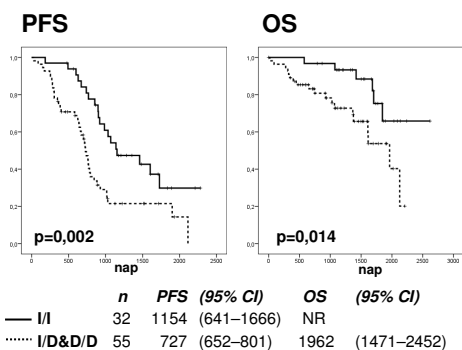


Figure 3. PFS and OS in VTD treated patients in the two NFKB1 genotype groups

According to the chemotherapy utilized, the biggest group was that of the bortezomib, thalidomide, dexamethasone (VTD) treated patients' [n = 88; 78 had and autologous bone marrow transplantation (ASCT) consolidation], here the PFS was 1154 days in the ins/ins and 727 days in the ins/del&del/del groups (p = 0.002), and in addition we also found a significant difference in the median OS, which was not reached in the ins/ins group (65.8% after 2617 days) and was 1962 in the ins/del&del/del group (p = 0.014) (Figure 3).

The analysis of the diagnostic delays

The mean and median times from first MM related symptom to diagnosis were 6.3 and 4.1 months, until the start of the first chemotherapy were 7.4 and 5.2 months respectively. Most patients

went to see their GP with bone pain, in this group the median waiting time to diagnosis was 4.5 months. Patients presenting with anaemia or renal failure had a quicker diagnosis: median 2.5 and 3.0 months respectively.

We could demonstrate any connections between the diagnostic delay and either PFS ($p = 0.399$) or OS ($p = 0.666$).

Conclusions

1. Our results showed no increased susceptibility to MM in either IL6 promoter -174G>C (rs1800795), or IL6 receptor Asp358Ala (rs2228145) genotype groups.
2. Similarly none of the NFKB1 -94ins/delATTG (rs28362491) variants increased the risk of MM.
3. Analyzing the survival of our 295 MM patients showed that the wild type variant (ins/ins) of the NFKB1-94ins/delATTG polymorphism had a significant positive effect on PFS but not on OS in the whole cohort.
4. This positive effect regarding PFS was even more pronounced in subgroups with better prognosis such as ISS 1+2, FISH standard risk and those having had bortezomib based treatment. The fact that in these subgroups the del carriers had a significantly worse outcome emphasize the importance of our findings, as this means that the NFKB1 genotyping can identify patients who are in the good prognosis subgroups according to the tests currently standard but in fact have a poor outcome in the presence of one or two del alleles.
5. Based on our findings the homozygous insertion genotype group benefits most from being treated with VTD plus ASCT which is the most effective combination currently founded in first line, while in the del carriers the outcome was poor even with this approach. The practical consequence could be that on the one hand in ins/ins patients it is worth pushing this expensive combination while in carriers of the del allele only clinical trials could improve the outcome.
6. Our study regarding the diagnostic delays and outcomes showed that in Hungary, similarly to other countries, patients with MM often wait far too long until finally get a diagnosis. This is in part the results of the fact that the typical presenting symptoms of myeloma (like back pain) are rather frequent in the general population but also the consequence of the weakness of the primary care system. As during these delays most patients were under the care of their primary physicians the solution should be on this level too. Guidelines, better outlined patient pathways could help to ameliorate the situation.

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