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THE ROLE OF SINGLE NUCLEOTIDE POLYMORPHISM OF IL-6 AND IL-10 CYTOKINE ON PAIN SEVERITY AND PAIN RELIEF AFTER RADIOTHERAPY IN MULTIPLE MYELOMA PATIENTS WITH PAINFUL BONE DESTRUCTIONS

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Multiple myeloma (MM) cells interact with bone marrow stromal cells stimulating transcription and secretion of cytokines like IL-6 and IL-10, which are implicated in the progression and dissemination of MM. Regulation of cytokines secretion is under genetic control through genetic polymorphisms in their coding and promoter sequences. It seems that single nucleotide polymorphism (SNP) in the promoter region of various genes may regulate the plasma concentrations of cytokines. Cytokines could be also hypothesized to function as pain modulators as peripheral nociceptors are sensitized by cytokines. The aim was to determine if the SNP of IL-6 and IL-10 cytokines could influence the analgesic response of radiotherapy in the treatment of painful bone destructions in MM patients. 30 patients (19 women and 11 men, median age: 67 years) with MM and painful bone destructions were treated with palliative radiotherapy. Pain was evaluated according to the visual analogue scale and analgesics intake. Pain scores and analgesics use were measured prior to radiotherapy as well as 4, 12 and 24 weeks afterward. Opioid

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analgesics were converted to the morphine-equivalent daily dose (MEDD). Genomic DNA was extracted from peripheral blood leukocytes and IL-6 and IL-10 gene promoter polymorphisms were analysed with polymerase chain reaction. 60% of patients reported severe pain prior to radiotherapy, which decreased to 13% at the first follow-up visit ($p < 0.001$). The MEDD on admission to the hospital was 75 mg/day which decreased to 46 mg/day at the first follow-up visit ($p = 0.033$). A significant parameter in pain relief was: age < 65 years ($p=0.029$). We analysed 6 SNPs in the gene promoter region of IL-6 (-597 G/A, -572 G/C, -174 G/C) and IL-10 (-592 A/C, -819 C/T, -1082 A/G) as well as their relation with pain severity and analgesic consumption. Patients who are IL-10 -1082 A/G carriers are prone to respond better to radiotherapy than other patients ($p<0.05$). A borderline association was noted for patients who are IL-6 -597 A/A and G/G carriers - assumed to be at higher risk for severe pain prior to radiotherapy ($p=0.07$) while for patients who are IL-10 -1082 A/A carries: the median pain score decreased faster ($p=0.08$). Patients with genotypes IL-6 -597 A/A and IL-6 -174 C/C required a smaller dose of opioids ($p=0.06$). SNP of IL-6 and IL-10 cytokines can influence the analgesic response of radiotherapy. Patients with genotype IL-10 -1082 A/G respond better to radiotherapy.

Key words: Multiple myeloma, single gene polymorphism, pain relief.

INTRODUCTION

Multiple myeloma (MM) is a clonal plasma cell neoplasm derived from an early precursor of B cell lineage characterized by anaemia, renal impairment, painful bone destructions and hypercalcaemia. The growth of MM cells is dependent on a complex interaction between growth factors, adhesion molecules, plasma cells and bone marrow stromal cells. MM cells interact with bone marrow stromal cells stimulating transcription and secretion of cytokines, which are implicated in the progression and dissemination of MM. The role of interleukin-6 (IL-6) and interleukin-10 (IL-10) is well established in MM pathogenesis (LAUTA 2001, ZHENG *et al*, 2008, KLEIN *et al,* 1999, LAUTA 2003, URBANSKA-RYS *et al*, 2000).

IL-6 is a cytokine produced by different cells: leukocytes, adipocytes, endothelial cells, fibroblasts, and myocytes. It plays a role in the pathogenesis of inflammation, autoimmune and lymphoproliferative diseases. IL-6, which is produced via an autocrine mechanism by MM cells itself, is one of the most important cytokines that contributes to MM cells survival and proliferation, whereas the paracrine mechanism is believed to be involved in the production of IL-6 in a microenvironment in the bone marrow (LAUTA 2001, LAUTA 2003). Production control of IL-6 may be exerted by other interleukins such as Interleukin-1beta (IL-1β) and IL-10 as well (ZHENG *et al,* 2008).

IL-10 is a cytokine produced by macrophages, T cells, B cells, natural killer cells and mast cells (ZHENG *et al.* 2001). IL-10 is described as the most potent factor of B-cell differentiation and an important growth factor of MM cells (LU *et al.* 1995) associated by the induction of an oncostatin M autocrine loop (Gu *et al.* 1996).

Increased serums levels of IL-6 and IL-10 are observed in MM patients. High serum level of IL-6 correlate with the stage of MM and degree of bone destruction (DUVILLARD *et al.* 1995). Thus, IL-6 is considered to be an important prognostic factor in MM patients and as

increased serum levels of IL-6 levels have been associated with a poor prognosis (LAUTA. 2003). High serum levels of IL-10 correlate with progressive MM disease (WIERZBOWSKA *et al.* 1999). Furthermore, higher serum IL-10 levels correlates with higher IL-6 levels in MM patients (URBANSKA-RYS *et al.* 2000).

Regulation of cytokine secretion is under genetic control through genetic polymorphisms in their promoter sequences. It seems that single nucleotide polymorphism (SNP) in the promoter region of the IL-6 gene at position -174 may regulate the plasma concentrations of IL-6 (BENNERMO *et al.* 2004, ENDLER *et al.* 2004). The CC genotype is considered to be a low producer and GG genotype a high producer of IL-6 (ZHENG *et al*, 2008, OLOMOLAIYE *et al.* 1998). The IL-10.G genotype 136/136 is associated with most increased secretion of IL-10 among patients with MM (ZHENG *et al*, 2001). But there was no relation between SNP at the position -1082, -819 or -592 and the disease expression (ZHENG *et al*, 2001, MAZUR *et al.* 2005).

Peripheral nociceptors are sensitized by cytokines that are produced in response to cancer or its cytostatic treatment. Cytokines released by activated glial cells, cause hyper excitability in pain transmitting neurons, increase the release of substance P and excitatory amino acids from presynaptic terminals triggering a high pain response (WATKINS *et al.* 2005, WATKINS *et al.* 1995, WATKINS *et al.* 1994). Cytokines could be also hypothesized to function as pain modulators. This is one of the main direct mechanisms leading to hyperalgesia in chronic disease (JUNGER *et al.* 2000). An analytic literature review revealed some studies that suggest variants in cytokines genes, which could explain the individual variations in reported pain severity and analgesic response among cancer patients (REYES-GIBBY *et al.* 2013, REYES-GIBBY *et al.* 2009, REYES-GIBBY *et al.* 2008, REYES-GIBBY *et al.* 2007, MCCANN *et al.* 2012, ILLI *et al.* 2012, SVETLIK *et al.* 2013, REYES-GIBBY *et al.* 2009, RAUSCH *et al.* 2010). The aim of this study was to determine if the SNP of IL-6 and IL-10 cytokines could influence the analgesic response of radiotherapy in the treatment of painful bone destructions in MM patients.

MATERIALS AND METHODS

Patient's characteristics

30 patients (19 women and 11 men, median age: 67 years, 51 – 83 years) with multiple myeloma and painful bone destructions were enrolled into the study, which was conducted at the Department of Oncology and Haematology at the Hospital of Lithuanian University of Health Science from September 2012 until September 2013. According to the Durie and Salmon staging system (DURIE *et al.* 1975) three patients (10%) with stage II and 27 (90%) patients with stage III MM (disease) were classified. Fifteen (50%) patients were identified with IgG type M protein, 7 (23%) patients for IgA type and 8 (27%) patients with light chains type MM. Inclusion criteria: patients diagnosed with symptomatic multiple myeloma, proved by histological data, electrophoresis and immunofixation of serum and urine as well as patients, with bone destructions verified by bone X-ray, computed tomography (CT) resulting in subjective pain perception or an impending fracture accompanied by a Karnofsky Index above 40 were included. Exclusion criteria: patients with bone metastases from solid tumors, patients with solitary plasmacytoma, patients who had previously received irradiation to the same site of destruction site, and patients of poor health state were excluded from the study. Patients with painful bone destructions were treated with palliative radiotherapy. They were randomized into two arms: 12 (40%) patients received 8 Gy in a single fraction regimen and 18 (60%) patients were treated

with 30 Gy in a 10 fractions regimen. Twenty two (73%) irradiated sites included the spinal column, 5 (17%) the pelvic bone and 3 (10%) the extremities. 28 patients (93%) were treated with concurrent chemotherapy. Surgery was performed prior to radiotherapy for 9 (30%) patients. Patient's characteristics are shown in Table 1a,b and c.

Table 1a.Patients characteristics.

Characteristics	N = 30	%
Sex		
Male	11	37
Female	19	63
Age (years)		
Range	51 - 83	
Median	67	
Clinical stage (Durie and Salmon)		
II	3	10
III	27	90
Karnofsky Index (%)	50 - 80	
Median	60	
Paraprotein		
IgG	15	50
IgA	7	23
Light chain	8	27

SE: standard error of mean

Table 1b.Patients characteristics.

Characteristics	N = 30	%
Irradiated sites		
Spinal vertebrae	22	73
Pelvic bone	5	17
Extremites	3	10
Surgery		
Yes	9	30
No	21	70
Concurrent chemotherapy		
Yes	28	93
No	2	7
Pain score at admission		
2-4	5	17
5-7	7	23
8-10	18	60
Pain medication	*%*	
Non-opioids	4	13
Opioids	26	87

SE: standard error of mean

Table 1c.Patients characteristics.

Characteristics	N = 30	%
Opioids dose (mg/day)		
Mean	71	
Range	10 - 260	
Radiotherapy regimen		
8 Gy one fraction	12	40
30 Gy in 10 fractions	18	60

SE: standard error of mean

Pain was evaluated according to the visual analogue scale (VAS) with scale endpoints ranging from 0 (no pain at all) to 10 (worst imaginable pain) (JENSEN *et al*. 1986). Pain scores ≤ 4 were classified as mild, 5 – 7 as moderate and ≥ 8 as severe (CHOW *et al*. 2006). Pain scores and analgesics use were measured prior to initiation of treatment and at 4, 12 and 24 weeks after radiotherapy. Medication was classified into two groups: non-opioids and opioids. Opioid analgesics were converted to the morphine-equivalent daily dose (mg/day) (SELBY and YORK PALLIATIVE CARE TEAM and PHARMACY GROUP, 2011).

Genotyping of IL-6 and IL-10 Genomic DNA was extracted from peripheral blood leukocytes with the help of an commercially available DNA extraction kit (GeneJet Genomic DNA Purification kit, Thermo Fisher Scientific), following the manufacturer‘s instructions. IL-6 and Il-10 gene promoter polymorphisms were analysed with polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP).

IL-6 gene region including -597 G/A and -572 G/C polymorphic sites were amplified by using primers reported by K. Snoussi et al. (SNOUSSI *et al*. 2005). Earlier described PCR conditions were slightly modified. Briefly, PCR reaction was carried out in a total volume of 25 µl containing 1x DreamTaq standard buffer, template DNA, 1.2 µM of each primer, 4.0 mM MgCl2, 200 µM of each dNTP and 0.65 U of DreamTaq DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA) with annealing at 58 °C. The amplicon was then digested either by FokI or MbiI restriction endonucleases (Thermo Fisher Scientific, Waltham, MA, USA) for the detection of -597 G/A and -572 G/C polymorphisms, respectively. The length of the fragments was as indicated by K. Snoussi et al. (SNOUSSI *et al.* 2005). The primer sequences for IL-6 gene -174 G/C polymorphism was as described by C.R. Duch et al. (DUCH *et al*. 2007). The PCR conditions were set according to the protocol presented above with different annealing temperature of 59 °C. NlaIII restriction endonuclease was used for Il-6 -174 G/C genotyping. The fragments were separated electrophoretically using 2% agarose gel containing ethidium bromide.

Il-10 gene promoter polymorphisms were analyzed as reported by J. Liu *et al* (LIU *et al*. 2011). For -819 C/T and -592 C/A polymorphisms the same reaction mixture composition was used. Briefly, PCR reaction was carried out in a total volume of 25 µl containing 1x DreamTaq standard buffer, template DNA, 0.24 µM of each primer, 200 µM of each dNTP and 1.25 U of DreamTaq DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA) with annealing at 58 °C and 63 °C for -819 C/T and -592 C/A polymorphisms, respectively. The PCR reaction conditions for Il-10 gene -1082 G/Apolymorphism were slightly modified by adding 4.0 mMMgCl2, 4% DMSO and changing anealing temperature to 54°C. Il-10 gene -1082 G/A, -819

C/T and -592 C/A polymorphisms were analyzed using MnlI, MaeIII and RsaI restriction endonuclease, respectively. The results were visualized on 3% agarose gel containing ethidium bromide.

Statistical analysis

Statistical data analysis was performed by using the IBM SSPS Statistics 21.0 for Windows (SPSS Inc., Chicago, IL, USA). Descriptive statistics were indicated to summarize patients' characteristics (Table 1). Chi squared test and Fisher's Exact test were used for identification of small expected frequencies permitting comparison regarding proportions of pain reduction among groups created by socio-demographic and clinical characteristics. The logistic regression was used to evaluate associations between severe pain status or pain relief, demographic and clinical variables and different genotypes (Table 2 and Table 3). The Chi squared test (Fisher's Exact test (or Chi square exact test) for small expected frequencies was used to compare pain severity proportions among different genotype groups. McNemar test was used to compare paired proportions of pain severity. The Mann-Whitney U test was used for evaluation of pain score differences and analgesic consumption between two independent genotype groups and the Kruskal-Wallis test was used for the three independent genotype groups.). Differences between compared characteristics were taken as statistically significant if p-value <0.05.

The study protocol was prepared in accordance with the Helsinki Declaration and was approved by the Lithuanian Regional Research Ethics Committee and State Data Protection Inspectorate.

RESULTS

Sixty percent (18/30) of the patients reported severe pain (>7/10) on admission to the hospital which decreased to 13% (4/30) after one month at the first follow up visit (McNemar p <0.001). The Mean Morphine Equivalent Daily Dose (MEDD) on admission to the hospital was 75 mg/day (range 10 – 260 mg/day, median 60) which decreased to 46 mg/day (range 0 – 140 mg/day, median 15) at the first follow up visit after radiotherapy (Wilcoxon p = 0.033). A significant parameter in pain relief was: age < 65 years (p=0.029). Other investigated parameters (sex, paraprotein, stage of disease, concurrent chemotherapy, Karnofski Index, hemoglobin level, radiotherapy dose) were insignificant.

Higher pain scores were more prevalent among younger patients (<65 years), in men than in women, among those with third stage of myeloma and with lower Karnofsky Index (<60%) as well as among those patients with IgA and light chains multiple myeloma (Table 2). Unfortunately results were statistically not significant due to low sample size. Greater pain relief was observed in patients with IgG type multiple myeloma, in men than in women, in patients treated with 30 Gy radiotherapy regimen, among patients with poor health status (Karnofski Index <60%) and as well as in those with a higher hemoglobin level (>10 g/dL). Due to low sample size results are not statistically significant (Table 3).

Table 2. Pain severity in patients with multiple myeloma.

Variable	Pain severity Severe/non severe	OR (95% CI)	p
Sex			
Male*	6/5	1.40 (0.24-8.16)	0.7
Female	12/7		
Age (years)			
<65	6/5	1.69 (0.21-13.75)	0.6
≥ 65*	12/7		
Clinical stage (Durie and Salmon)			
II*	1/2	3.87 (0.24-63.15)	0.3
III	17/10		
Karnofsky Index (%)			
<60%	9/3	3.8 (0.55-26.43)	0.2
≥60%*	9/9		
Paraprotein			
IgG*	9/6	1.55(0.24-10.11)	0.6
IgA and light chain	9/6		

*Reference group

Table 3.Pain relief in patients with multiple myeloma

Variable	OR (95% CI)	p
Sex		
Male	1.09 (0.08-14.16)	0.9
Female*		
Radiotherapy regimen		
30 Gy	5.28 (0.57-48.74)	0.14
8 Gy*		
Hemoglobin level (g/dL)		
≤10*	1.83 (0.16-20.81)	0.6
>10		
Karnofsky Index (%)		
<60%	1.0 (0.12-8.13)	0.9
≥60%*		
Paraprotein		
IgG	1.55(0.16-14.99)	0.7
IgA and light chain*		

*Reference group

We analysed 3 SNPs (-597 G/A, -572 G/C, -174 G/C) in IL-6 gene promoter region and 3 SNPs (-592 A/C, -819 C/T, -1082 A/G) in IL-10 gene promoter and their association with pain severity and analgesic consumption. Most patients were GG carriers in IL6 all genotyped

promoter positions. The majority of patients were CC carriers at position -592 and -879 and AG carriers at position -1082 in IL-10. No significant association between pain severity and different genotypes could be found due to a small sample size. An borderline association was evident for patients who are IL6 -597 A/A and G/G carries as these are at higher risk of severe pain before radiotherapy (p=0.07). All patients with IL6 -572 G/C genotype were at higher risk of severe pain, unfortunately no link between this particular genotype and pain could be established as it was determined only in two patients.

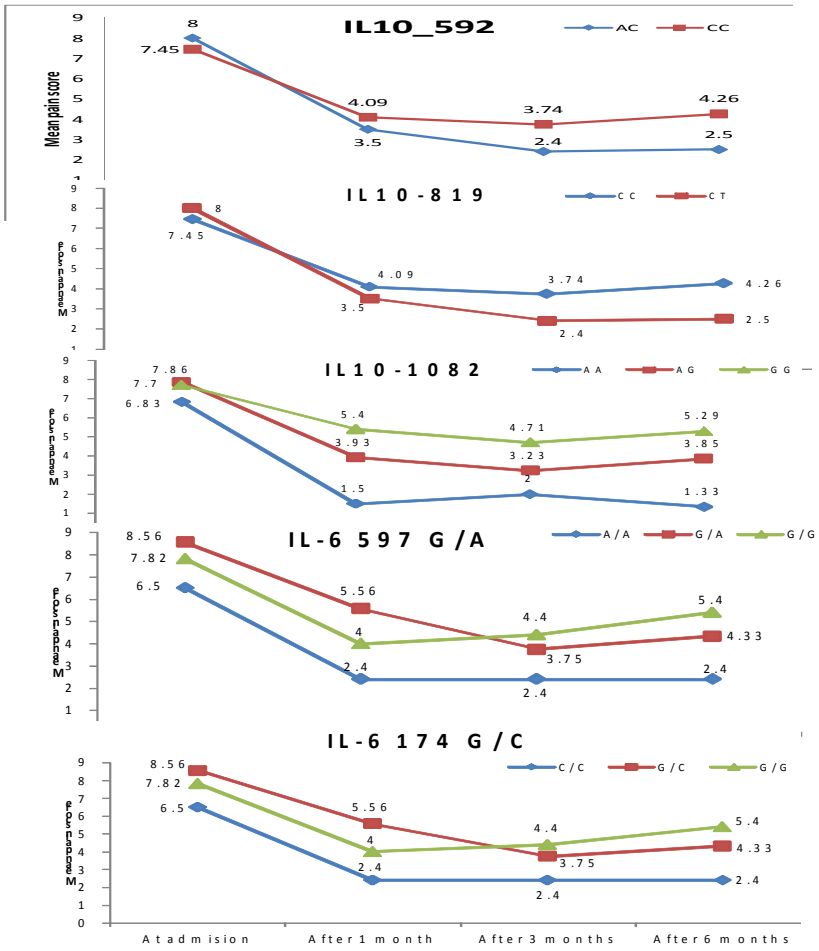

Figure1A. Pain severity in the patients with different genotypes at admission and in follow up

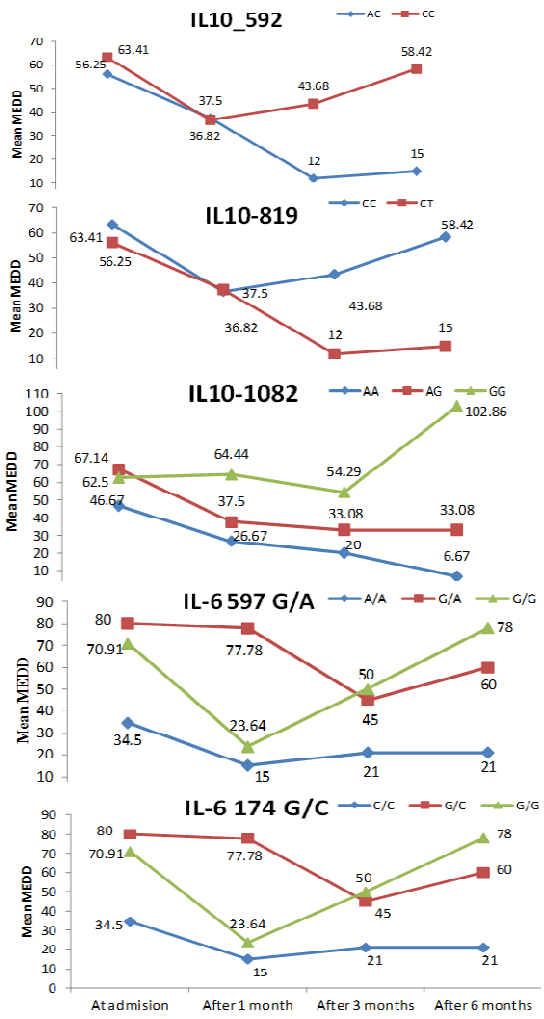

Figure 1B. Analgesic consumption at admission and after radiotherapy in the patients with different genotypes.

In Figure 1A you can see how pain severity decreased in the patients with different genotypes. The Mann-Whitney U and Kruskal-Wallis tests were used for evaluation of different pain severity scores between different genotype groups on admission to the hospital and one, three and six months after radiotherapy. We did not find a statistical significant difference regarding pain scores on admission and during follow up. Nevertheless a borderline association

of improvement of pain score for patients who are IL10 -1082 A/A carries was found: the median pain score decreased faster in those than in other patients at first month after radiotherapy (p=0.08). The patients with SNP of IL6 -572 G/C are not shown in the plot as there were only two patients with genotype IL6 -572 G/C and they died three weeks after the follow up.

The plots in Figure 1B shows analgesic consumption at admission and their decrease at one, three and six months after radiotherapy in patients with different genotypes. Unfortunately no statistical significant difference was found due to a small sample size but a tendency with borderline significance was noted as patients with genotype IL-6 -597 A/A and IL-6 -174 C/C required a smaller opioid dose for pain relief (p=0.06 and p=0.06).

Assessment between radiotherapy response and different patient genotype revealed that patients who are IL-10 -1082 A/G carries are prone to respond better to radiotherapy than other patients ($p<0.05$). However, no improvement in the response to radiotherapy was exhibited by other genotypes.

DISCUSSION

It is well know that pain perception, pain relief and analgesic consumption are highly individual. Sociodemographic characteristics (age, gender, race) and clinical variables (stage of disease, performance status, depressed mood, comorbid conditions) may modify individual pain sensation and the response to analgesic treatment. Several mechanisms can be involved in pain perception and relief: drug metabolized enzymes, transporters, receptors, proinflamatory cytokines and their impact on genetic variability. Peripheral nociceptors are sensitized by cytokines that are produced by inflammatory cells (CD4+ and CD8+ T cells) in response to diseases as cancer or its treatment with cytostatics (SVETLIK *et al*. 2013). This is one of the main mechanisms leading to pain hypersensitivity in chronic diseases (JUNGER *et al*. 2000). Some studies demonstrate the impact of SNPs in genes encoding for these cytokines on pain severity and pain control in the treatment of various diseases (REYES-GIBBY *et al*. 2013, REYES-GIBBY *et al*. 2009, REYES-GIBBY *et al.* 2008, REYES-GIBBY *et al*. 2007, MCCANN *et al.* 2012, ILLI *et al.* 2012, SVETLIK *et al.* 2013, REYES-GIBBY *et al*. 2009, RAUSCH *et al*. 2010, RAMONDA *et al*. 2013, MIYASHITA *et al*. 2012, LOO *et al*. 2012). The current study assessed the impact of selected SNPs of IL-6 and IL-10 promoter gene on pain severity in patients with painful bone destructions due to multiple myeloma and pain treatment with palliative radiotherapy. We found out that patients who were IL-10 -1082 A/G carries responded better to radiotherapy than patients with other different genotypes ($p<0.05$) and that there was a borderline association for patients with genotype -1082 A/A in the IL-10 promoter gene, who are prone for faster pain relief (in the first four weeks) after performed radiotherapy (p=0.08). According to literature our study is the first one to give preliminary evidence that SNPs in cytokine genes associated with pain syndrome in multiple myeloma patients play a significant role in respect to radiotherapy response.

Studies show that about 40% of patients who are treated with active cancer treatment report pain which increases up to 80% for patients with advanced stages of cancer (REYES-GIBBY *et al*, 2009, BRUERA *et al.* 2000, ELSAYEM *et al*. 2004). REYES-GIBBY *et al.* observed that patients with lung and pancreas cancer with advanced stage of disease and patients at younger age were more likely to report severe pain related to their counterparts (REYES-GIBBY *et al* 2009, REYES-GIBBY *et al* 2007, REYES-GIBBY *et al* 2009). Depressed mood and fatigue significantly correlated with pain with different cancers (REYES-GIBBY *et al* 2009, REYES-GIBBY *et al* 2007, REYES-GIBBY

et al 2009, STEPANSKI *et al* 2009). Consisted with other studies we found that sociodemographic, clinical characteristics and genetic factors may be relevant in the severity of pain and pain relief. Due to a small sample size our study results were not statistically significant, but we observed that severe pain was more prevalent among patients younger than 65 years old, in men than in women, in those with third stage of MM, with a Karnofsky Index lower 60% and among patients with IgA and light chains MM. We also noted a tendency of higher pain relief in patients with IgG type MM, more in men than in women, in patients treated with 30 Gy radiotherapy regimen, among patients with poor health status (Karnofski Index <60%) and in those with a higher hemoglobin level (>10 g/dL).

Studies suggested that proinflammatory cytokines released by activated glial cells, during inflammation or tissue damage modify the activity of nociceptors leading to pain hypersensitivity (WATKINS *et al*. 2005, WATKINS *et al*. 1995, WATKINS *et al*. 1994). A marginal significance was noted in our study that multiple myeloma patients who were IL-6 -597 A/A and G/G carries had a higher risk of severe pain prior to radiotherapy (p=0.07). In our study there were two patients with IL-6 -572 G/C genotype and both of them perceived high pain scores. Meanwhile REYES-GIBBY *et al*. did not find the relationship between IL-6 genotypes and pain severity for patients with lung and pancreas cancers (REYES-GIBBY *et al*. 2008, REYES-GIBBY *et al*. 2009). Variant alleles in TNF-α -308 G/A were significant associated with severe pain in a study with lung cancer patients (REYES-GIBBY *et al*. 2009, REYES-GIBBY *et al*. 2008), but not in adenocarcinoma of the pancreas (REYES-GIBBY *et al*. 2009). Illi et al. did not find the relation between SNPs of TNF-α and IL-6 cytokines in their study and the following symptoms including cluster of pain, fatigue, sleep disturbance and depression in oncology patients (ILLI *et al*, 2012). Others studies found that IL-8 -251 T/A were significantly associated with severe pain in patients with lung and pancreas cancers (REYES-GIBBY *et al*. 2013, REYES-GIBBY *et al*. 2007, REYES-GIBBY *et al*. 2009]. A study about lung cancer patients of Rausch et al. found significant associations with rs10800871 of IL-10 and the severe pain (RAUSCH *et al* 2010). However in others studies the relationship between IL-10 genotypes and pain severity for patients with adenocarcinoma of the pancreas and lung cancer was not found (REYES-GIBBY *et al*. 2013, REYES-GIBBY *et al*. 2009)

Proinflammatory cytokines released by activated glial cells, but also opioids increase glial production of proinflammatory cytokines, which excite pain responsive neurons and counterbalance analgesia by creating compensatory pain relief (REYES-GIBBY *et al*. 2008). Thus effects of cytokines on pain can be bidirectional: increase and facilitation of pain syndrome as well as increased opioid tolerance. One study have found out that carriers of the IL-6 -174 C/C genotypes required a 4.7 times higher dose of opioids for pain relief related to GG and GC genotypes (p=0.004) and no statistically significant tendency for higher MEDD by polymorphisms in IL-8 (REYES-GIBBY *et al*. 2008). Controversy our findings suggest a marginal significance that patients with IL-6-174 C/C and IL-6-597 A/A genotypes required lower doses of opioids (p=0.06). The IL-6 -174 G/C polymorphism affects transcription, altering serum levels of IL-6 with the C allele associated with significantly lower levels of plasma IL-6 (FISHMAN *et al* 1998, TERRY *et al*. 2000). Our preliminary findings provide that patients who are homozygous for the allele associated with lower levels of IL-6 and require lower doses of opioids. Some studies showed that IL-6 enters the systemic circulation, where its concentration correlates with the severity of injury and subsides days after (DE JONGH *et al*. 2003).

There are limitations in our study: we have analysed SNPs contribute to pain severity and pain relief, but did not valuated symptoms like depression mood and fatigue, which are very common in patients with MM and which may have an impact on pain syndrome. We assessed only several SNPs of two cytokines genes, there is more genetic variation for each gene, without including multiple SNPs we also did not analysed haplotypes, which may give different outcomes. We do acknowledge and agree that there is an interaction between different cytokines and other genes with functional significance, which requires more precise evaluation.

In conclusion, despite the great progress in pain treatment and management of multiple myeloma patients, there are a lot of patients suffering from severe pain. Sociodemographics characteristics, clinical and psychological factors influence pain and its treatment. Our study gives the preliminary evidence that the SNPs in cytokine genes correlate with pain syndrome in multiple myeloma patients and analgesic response to radiotherapy, thus it may be useful to develop risk models for better understanding of pain, which permits a better and more individualized therapy for MM patients. Future prospective studies with large sample of patients incorporating additional genetic markers are needed to validate our findings.

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ULOGA POLIMORFIZMA JEDNOG NUKLEOTIDA IL-6 I IL-10 CITOKININA U OTKLANJANJU BOLOVA POSLE RADIOTERAPIJE PACIJENATA SA MULTIPLIM MIELOMOM (MM) KOJI IZAZIVA BOLNU DESTRUKCIJU KOSTI

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Izvod

Regulacija sekrecije citokinina je pod genetičkom kontrolom preko polomorfizma kodirajuće i promoterske sekvence. SNP (polimorfizam jednog nukleotida) u region promoter različitih gena može da regulše koncentraciju citokinina u plazmi. Vršena su istraživanja da li SNP u IL-6 i IL-10 citokinina može da utiče na bolnu reakciju posle radioterapije pri tretmanu bolne destrukcije kosti kod MM pacijenata. Vršena su ispitivanja 6 SNPs u region promoter gena IL-6 (-597 G/A, -572 G/C, -174 G/C) i IL-10 (-592 A/C, -819 C/T, -1082 A/G) kao i odnos sa različitim bolovima i korišćenim lekovima. Zaključeno je da SNP u IL-6 i IL-10 citokina može da utiče na odgovor bola posle radioterapije. Pacijenti sa genotipom IL-10 -1082 A/G reaguju bolje na radioterapiju.

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