



AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Chemotherapy-Induced Neurotoxicity: Evidence of a Protective Role of CC Homozygosity in the Interleukin-1 β Gene-511 C>T Polymorphism**This is the author's manuscript***Original Citation:**Availability:*This version is available <http://hdl.handle.net/2318/1619721> since 2017-01-17T14:48:42Z*Published version:*

DOI:10.1007/s12640-016-9637-1

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



This is the author's final version of the contribution published as:

Peila, E.; D'Agata, F; Caroppo, P.; Orsi, L.; Mortara, P.; Cauda, S.; Manfredi, M.; Caglio, M.M.; Fenoglio, P.; Baudino, B.; Castellano, G.; Bisi, G.; Pinelli, L.; Gallone, S.. Chemotherapy-Induced Neurotoxicity: Evidence of a Protective Role of CC Homozygosity in the Interleukin-1 β Gene-511 C>T Polymorphism. NEUROTOXICITY RESEARCH. 30 (3) pp: 521-529.
DOI: 10.1007/s12640-016-9637-1

The publisher's version is available at:
<http://link.springer.com/10.1007/s12640-016-9637-1>

When citing, please refer to the published version.

Link to this full text:
<http://hdl.handle.net/2318/1619721>

**Chemotherapy-induced neurotoxicity: evidence of a protective role of CC
homozygosity in the Interleukin-1 β gene -511 C>T polymorphism**

Peila E^{a*}, D'Agata F^{a*}, Caroppo P^{ab}, Orsi L^a, Mortara P^a, Cauda S^c, Manfredi M^c,
Caglio MM^a, Fenoglio P^a, Baudino B^c, Castellano G^c, Bisi G^c, Pinessi L^a, Gallone S^a

a Department of Neuroscience, AOU San Giovanni Battista, Turin, Italy

b IRCCS Fondazione Istituto Neurologico Carlo Besta, Milan, Italy

c Nuclear Medicine, AOU San Giovanni Battista, Turin, Italy

* Peila E and D'Agata F equally contributed to this work

 Corresponding author: Federico D'Agata

Neurosciences Department, Via Cherasco, 15 - 10126, Turin, Italy

Tel: +390116636298; Fax: +390116963487

federico.dagata@unito.it

Running title: Protection of CC IL-1 β -511 from chemotherapy neurotoxicity

Abstract: 141 words, Main text: 3348 words, Figure: 0, Tables: 8.

The authors declare no conflict of interest.

Abstract

Objectives: We hypothesized that the IL-1 β -511 C>T polymorphism could be associated with the development of neurotoxicity and that it could be a possible biomarker to rate the risk of occurrence of neurotoxicity in cancer patients.

Methods: Genomic DNA was extracted from 85 cancer patients: 49 received systemic chemotherapeutic treatment (CHT) and 36 patients did not receive it (No-CHT). All subjects were genotyped for the functionally active polymorphisms of IL-1 β -511 C>T. We estimated neurotoxicity with the evaluation of neurological deficits.

Results: CHT patients showed erythrocytopenia, neurological deficit and a slight lowering of cognitive performance. The subgroup of patients carrying the CC genotype of the IL1 β -511C>T gene showed lesser neurological deficits.

Conclusion: In the context of cancer treatment, we suggested the potential value of IL-1 β -511 C>T as genetic biomarkers to identify patients with higher risk to develop neurological deficits.

Keywords: cytokines; IL-1 β ; neurotoxicity; chemotherapy; cancer

Introduction

Chemotherapy can allow long-term survival for patients with cancer, but neurologic side effects of chemotherapeutic drugs (neurotoxicity) are common and they are second in frequency (up to 60% of prevalence) only to hematological toxicity (Windebank and Grisold 2008). Neurologic side effects of chemotherapy can be classified as: peripheral neuropathy; cognitive impairment; fatigue and motivational deficits (Vichaya et al. 2015).

Peripheral neuropathy represents one of the most severe complications (Seretny et al. 2014). The typical clinical manifestations are pure sensory symptoms, sometimes sensory-motor, often associated with pain (Hausheer et al. 2006; Windebank and Grisold 2008; Argyriou et al. 2012).

The main cognitive domains involved in neurotoxicity are attention, memory, executive functions and processing speed (Cleeland et al. 2003; Baudino et al. 2012; O'Farrell et al. 2013). The cognitive changes have been associated with corresponding alterations in the brain integrity, perfusion or metabolism, generally reversible after years, (Inagaki et al. 2007; Baudino et al. 2012; Ganz et al. 2013).

Neurotoxicity effects can be acute or chronic after multiple exposures, in a small, although significant portion of patients, the effects could persist after treatment discontinuation (Argyriou et al. 2012; Vichaya et al. 2015; Wang et al. 2015).

Even if several mechanisms of neurotoxicity have been proposed, the pathogenesis has not been fully understood and can differ among classes of chemotherapeutic agents (Pettersson et al. 1990; Cavaletti et al. 2000). Many authors proposed that inflammatory cascade activation, pro-inflammatory cytokine up regulation and neuro-immune pathways play an essential role in the initiation and progression of neurotoxicity (Wang et al. 2012; Vichaya et al. 2015; Wang et al. 2015).

However, despite the use of the same chemotherapeutic drugs, not all patients present neurotoxicity, and several studies have been focused on identification of risk factors, predictors of onset or long term persistence (Wilkes 2007; Argyriou et al. 2008a; Argyriou et al. 2008b; O'Farrell et al. 2013) including age, sex, lower education, alcoholism and comorbidities (Smith et al. 2008; Cavaletti and Marmiroli 2010; O'Farrell et al. 2013). Several authors investigated the individual susceptibility to develop neurotoxicity through pharmacogenomics studies. Up to now, several Single Nucleotide Polymorphisms (SNPs) have been described as susceptibility factors in peripheral neuropathy (Cavaletti et al. 2011) and could partly explain the individual differences observed.

Interleukin 1 beta (IL-1 β) represents a powerful mediator of the inflammatory response. Its gene belongs to the complex of *IL-1* gene, mapped to the chromosome 2q13-21 and encodes IL-1 α , β and Ra proteins. IL-1 α and β are pro-inflammatory proteins, whereas IL-1Ra has an anti-inflammatory function (Dinarello 1994; Nicklin et al. 1994). The balance between IL-1Ra and IL-1 β is the most relevant factor to determine the inflammatory response (Arend 2002).

IL-1 β gene has a CT bi-allelic polymorphism in the promoter region at position -511 (di Giovine et al. 1992). To our knowledge the role of IL-1 β polymorphisms in the occurrence of neurotoxicity has never been investigated before. Here, we hypothesized that the IL-1 β -511 C>T polymorphism could be associated with the development of neurotoxicity and that it could be a possible biomarker of risk of occurrence of neurotoxicity in cancer patients. In addition, the possible effect of IL-1 α -889 C>T has also been tested.

Methods

A single nucleotide polymorphism (SNP) is a variation of in a single nucleotide that occurs at a specific position in the genome, where each variation is present to some appreciable degree within a population and most human SNPs are biallelic.

We used the polymerase chain reaction (PCR), a technique that amplify across several orders of magnitude a few copies of DNA, to determine the SNP frequency of IL1 β - 511 C>T and IL-1 α -889 C>T polymorphisms in our sample.

Patients

Eighty-five cancer patients (39 males, 46 females, mean age 53, SD 14, all right-handed) with haematological or solid tumours, were recruited among a group of subjects undergoing a PET study at the Medicine Nuclear Center of University of Turin, Italy (Baudino et al. 2012). Patients were enrolled among those who were planned to undergo a whole-body [18F]FDG PET on a clinically routine basis for hematological or solid tumor staging or to monitor the disease after treatment. Subjects were considered eligible, if they did not have neurological and psychiatric disorders, systemic diseases (HIV, diabetes) or medications that could potentially alter the neuropsychological performances and/or brain metabolism. Patients with history of peripheral neuropathy (hereditary or paraneoplastic) were excluded.

Eligible patients gave their written informed consent to participate to the project, which was approved by the local ethical committee according to the Helsinki Declaration (Comitato Etico Interaziendale of AOU Città della Salute e della Scienza of Turin - San Giovanni Battista University Hospital, number [166-179-70-2009]).

Patients were divided in two groups: 49 patients received systemic chemotherapeutic treatment (CHT patients) and 36 patients did not receive it or were waiting to start chemotherapy (No-CHT patients) and were considered as a control group. The 72% of

chemotherapy treated patients and none of the non-chemotherapy patients were disease-free.

All the patients were Italians and of Caucasian ethnicity.

Neurological and Neuropsychological assessment

All patients and controls have been evaluated by a complete neurological examination by an expert neurologist blinded to the chemotherapy status of the patient.

The following tests were performed: complete interview, cranial nerves testing, motor function evaluation (muscle tone and strength evaluation, gait, posture), sensory function evaluation (pain and temperature sensation, proprioception, light touch), reflexes, cerebellar function evaluation (finger to nose test, tandem walking, Romberg test).

Neurological findings were divided in 7 domains: consciousness, speech impairment, motor function, sensory function, balance/gait, reflexes and cranial nerves. A score had been assigned to each domain according to the NCI-CTC method (National Cancer Institute - Common Toxicity Criteria), ranging between 0 (normal) and 4 (life-threatening or disabling adverse event). For neuropathy assessment the NCI-CTC scale considered: 1 Asymptomatic: loss of deep tendon reflexes or paresthesia 2 Moderate symptoms: limiting instrumental activities of daily living 3 Severe symptoms: limiting self-care activities of daily living 4 Life-threatening consequences. Data had been binarized for each domain and for total neurological examination (0-normal; 1-deficit). One deficit in one domain was sufficient to consider the domain as impaired.

Assessment of Cognitive Domains

The patients were evaluated with a standardized neuropsychological assessment consisting of a battery of cognitive tests involving many domains. We used the standardized Italian versions of tests with normative values for the Italian population (Spinnler and Tognoni 1987). The tests scores were corrected for age and education.

The battery included:

- Mini Mental State Examination (MMSE), a brief 30-point questionnaire test (min 0, max = 30) used to screen for cognitive impairment (< 24); it includes tests of orientation, attention, memory, language and visual-spatial skills;
- Phonemic Fluency, that requires to retrieve as many as possible F-, A- and S- beginning words in 3 consecutive 1-min time periods, excluding perseverations or out-of-category words (min 0, max = number of words);
- Short Story Test (SST), a verbal memory test that require to recall meaningful verbal material and content idea from a listened short story immediately and after 10-minute delay (min = 0, max = 16);
- Trail Making Test B (TMT B). provide a measure of attentional-executive functioning recording the seconds needed to connect 25 circles in an ascending sequence of alternating numbers and letters (e.g., 1, A, 2, B, etc...).

Anxiety, depression and distress through were also assessed by self-report questionnaires:

- Distress Thermometer (DT) is a visual analog scale asking the patient to describe his level of distress during the last week on a 0 (no distress) to 10 (extreme distress) scale. The cutoff score used to identify ‘distressed’ persons is 4;
- Hospital Anxiety and Depression Scale (HADS) a 14-items (each item rated 0–3) self-report scale to measure anxiety (HADS-A, 7 items, range score 0–21)

and depression (HADS-D, 7 items, range score 0–21). A cut-off ≥ 8 indicates cases of clinical anxiety and depression.

The cognitive and neurological evaluations of the CHT patients were assessed at variable time after the end of the chemotherapy, but in most cases after 12 months (range 1-133 months).

Genetic analysis

Genomic DNA was extracted from 200 μ L of peripheral blood using the ISOLATE II GENOMIC DNA Kit (BIOLINE). The IL-1 β -511 SNP was genotyped in patients as previously described (di Giovine et al. 1992).

The determination of the IL-1 α -889 C>T, IL-1 β -511 C>T biallelic polymorphisms were based on a restriction-fragment length polymorphism analysis, as described elsewhere (Wilson et al. 1992; Huang et al. 1998; Huang et al. 1999; Grimaldi et al. 2000). Table 1 showed the DNA primers, the conditions of PCR and digestion patterns. PCR reactions were performed in a final volume of 25 μ L, with 25 ng of genomic DNA, 0.15 unit of MYTaq DNA polymerase HS (BIOLINE), 125 nM of each primer, 1.5 mM MgCl₂ and 50 mM dNTPs. The following PCR conditions, an initial denaturation at 95°C for 5 min and 35 cycles at 95°C for 30 s, specific T annealing for 30 s, 72°C for 40 s, and a final elongation at 72°C 10 min were performed. PCR products were electrophoresed on a 1.5% agarose TBE 1X gel and stained with ethidium bromide. The amplified fragment was digested with restriction enzymes (MBI Fermentas). The incubation was performed at 37°C for 4 h and the fragments were separated on 2.5% agarose TBE 1X gel.

Statistical analysis

All statistical analyses were performed with IBM SPSS Statistics for Windows, Version 20.0.0 (Armonk, NY: IBM Corp.).

A descriptive and main statistical analysis was performed on the overall cohort of patients. Continuous variable data was described with mean and standard deviation (SD) or median and interquartile range (IQR) as appropriate on statistical distribution; categorical variable data was represented with frequency and percentage.

For comparison between groups (CHT vs. No-CHT, interleukins SNPs) we used t-test or Mann-Whitney U for continuous variables and a Chi-square or Fisher's exact test for categorical variables. All statistical tests were two-sided and p values < .05 were considered statistically significant.

Results

Demographic and clinical data were reported in Table 2, neuropsychological and psychological data in Table 3.

Most of patients (71%) had a Hematologic malignancy; a minority a Solid cancer (29%) with similar proportions in the CHT and No-CHT groups (Table 2). All selected patients were evaluated and treated in the Oncology Departments of the AOU Città della Salute e della Scienza (Turin, Italy).

The Hematologic malignancies were prevalently Non-Hodgkin's lymphomas (68%), the remaining were Hodgkin's lymphomas. The solid cancers were distributed as follow: breast (40%), duodenal-rectal (20%), lung (20%) and other (20%).

The CHT group underwent to conventional standard-dose chemotherapy. Table 4 summarized the cancer distributions and the administered chemotherapy protocols.

The ABVD (Adriamycin/Hydroxydaunorubicin/Doxorubicin, Bleomycin, Vinblastine and Dacarbazine) and the CHOP (Cyclophosphamide, Hydroxydaunorubicin,

Oncovin/Vincristine and Prednisone or Prednisolone) chemotherapy protocols were used in most of Hematologic patients (69%); in 56% of the cases the CHT has been associated with the monoclonal antibody Rituximab. The Epirubicin (24%), Fluorouracil (21%), Cyclophosphamide (14%), Taxanes (14%) and Platinum-based (7%) were the most used chemotherapy drugs in the Solid cancers group (see Table 4). CHT patients compared to No-CHT patients were younger ($p = .003$, Table 2), significantly differed for erythrocytopenia ($p = .006$, Table 2), neurological deficits ($p = .004$, Table 3) and, less strongly, for cognitive performance (MMSE $p = .044$, phonemic fluency $p = .041$, Table 3).

Alterations in one or more neurological domains were more frequently observed in CHT patients (Table 3).

No other significant differences have been observed between the two groups.

As shown in Table 5, all investigated polymorphisms were equally distributed in CHT and No-CHT patients. In our study, the polymorphisms were in Hardy-Weinberg equilibrium and did not differ from the distributions of healthy control subjects (Poli et al. 2002; Cavallone et al. 2003; Seripa et al. 2003).

Comparing the different polymorphisms we found significant associations for the IL-1 β and neurological effects in the CHT group (Table 6). The CC haplotype of IL-1 β was associated with lesser neurological deficits. This association was not observable in the No-CHT patients (Table 6). We did not find associations with age, education, depression, cognitive impairment (MMSE), time from chemotherapy, or number of cycles and IL-1 β .

No association has been found for the IL-1 α -889 C>T.

Reflexes and Sensory function (paresthesia, dysesthesia, hypoesthesia) were more frequently impaired in CHT (Table 7), and in particular for the CT/TT haplotypes

subgroup (Table 8), even if association was statistically significant only for CHT and reflexes (Table 7).

Discussion

The present study considered a cancer group with high heterogeneity of treatments and pathologies in order to investigate the possible presence of a shared biological mechanism in the pathogenesis of neurotoxicity. The existence of similar mechanisms could partly explain the cross evidences of similar chemotherapy-induced changes in different cancer populations.

The patients that received adjuvant chemotherapy showed a worse cognitive profile, with lowering in frontal and general functioning. They also present a hematopoietic damage and a greater occurrence of peripheral neurological deficits. All these changes, are induced by chemotherapy, as reported in the literature (Windebank and Grisold 2008; Vichaya et al. 2015).

In the present study we hypothesized an association between IL-1 β -511 SNP and the incidence of chemotherapy-induced neurotoxicity; our hypothesis has been confirmed in a group of cancer patients treated with different categories of chemotherapeutic agents.

The subgroup with the CC allele in the IL-1 β -511 SNP had a minor occurrence of neurological deficits. Hence, this preliminary observation suggests that IL-1 β -511 SNP could be a marker of susceptibility to chemotherapy-induced neurotoxicity and a possible protective role of the CC homozygosity in the IL-1 β gene -511 C>T polymorphism could be suggested.

Possible pathological mechanisms involved in peripheral neurotoxicity have been extensively observed with evidences showing specific direct toxic mechanisms related to each chemotherapeutic class (e.g. mitochondrial functional impairment, DNA

duplication or transcription errors, neurotransmitter deregulation, oxidative stress, neurotrophic factors deregulation, microtubule-based axonal transport interruption, small fiber loss, hypothalamic-pituitary axis activation, (Windebank and Grisold 2008; Argyriou et al. 2012; Wang et al. 2012; Cavaletti and Marmiroli 2015; Vichaya et al. 2015; Wang et al. 2015)).

Some indirect mechanisms are responsible for the maintenance of neurotoxicity after drug discontinuation in 30-35% of patients (Argyriou 2012 et al.); in particular, inflammatory cascade activation, pro-inflammatory cytokine up regulation and neuro-immune pathways seem to play an essential role in the initiation, progression and maintenance of peripheral neurotoxicity (Wang 2012 et al.).

Despite these evidences, results from pharmacogenomics studies are still unable to identify clear genetic risk factor or prevention therapies for peripheral neurotoxicity (Cavaletti 2011 et al.; Hausheer 2006 et al).

The mechanisms of a possible chemotherapy central neurotoxicity are much less explored and not yet understood, but many evidences highlighted the possible central role of activation of inflammatory cascade and neurodegeneration (Ahles et al. 2002; Cleeland et al. 2003; Natori et al. 2015; Vichaya et al. 2015; Wang et al. 2015).

Moreover, inflammatory responses, partially related to deregulation by the neuroendocrine system, may mediate the onset of behavioral symptoms in cancer patients.

IL-1 β represents a powerful mediator of the inflammatory response and has an important role in the regulation of homeostatic mechanisms in peripheral nerve function and its role in neuropathic pain and central pain sensitization has been highlighted (Skundric et al. 2002; Sommer and Kress 2004; Ren and Torres 2009; del Rey et al.

2012). Furthermore, high levels of IL-1 β have been found (Forlenza et al. 2009; Miller et al. 2011) in blood and cerebrospinal fluid of patients with schizophrenia and Alzheimer Disease (AD). However, the contribution of IL-1 β in the genesis of neurotoxicity has been poorly investigated so far.

The T variant IL-1 β gene has a CT bi-allelic polymorphism at -511 position that has been associated to gastric and hepatic cancers (El-Omar et al. 2000), inflammatory diseases such as rheumatoid arthritis (Shafia et al. 2014) and diabetic nephropathy (Stefanidis et al. 2014). Of particular interest is the work of Bower et al. (Bower et al. 2013), a genomic investigation on 171 early breast cancer patients, where the authors found an increased genetic risk index associated with chemotherapy-induced fatigue over TNF- α -308, IL-6 -174, and IL-1 β -511 SNPs. In our work, we found similar results for neurological deficits and IL-1 β -511 SNP that could be considered a marker of susceptibility to chemotherapy-induced neurotoxicity.

Conflicting data are reported about the functional effect of this polymorphism on IL-1 β production (Pociot et al. 1992; Santtila et al. 1998). The authors suggested that the known allelic presentation of the IL-1 β gene is not a direct regulator of the IL-1 β production, and conversely, the IL-1RA allele 2 is associated to an elevated IL-1 β production *in vitro* (Santtila et al. 1998). However, other authors described enhanced IL-1 β circulating levels *in vivo* in patients with T allele at -511 position (El-Omar et al. 2000; Hall et al. 2004; Henderson and Poirier 2011).

The protective effect of CC genotype of IL-1 β -544 SNP in neurodegeneration has been already proposed by some authors (Licastro et al. 2000; Green et al. 2002; Di Bona et al. 2008; Déniz-Naranjo et al. 2008; Henderson and Poirier 2011).

Particularly, Naranjo et al. highlighted the central role of inflammation in neurodegeneration induced by T allele in IL-1 β -511 SNP (Déniz-Naranjo et al. 2008). They suggested that the neuro-inflammatory process leading to neurodegeneration could be initially mediated by pro-inflammatory activity of IL-1, which is overexpressed by activated microglia. The induction of pro-inflammatory molecules, and related signalling pathways, could lead to both synaptic and neuronal damage, as well as to further inflammatory cells activation; the increase of activated microglia leads to additional IL-1 release in a self-sustaining loop (Griffin 2006; Weisman et al. 2006). The immune and nervous systems can communicate and reciprocally influence each other regulating the inactive and reactive states of glial cells. Microglial activation, following CHT, causes the release of pro-inflammatory cytokines such as IL-1, IL-6, and TNF- α . Although the function of these molecules is to prevent neural damage, they could become toxic to nervous system's cells (Smith et al. 2012). In fact, excessive production of cytokines, such as IL-1, that are neuroprotective in low concentrations, could cause neurodegeneration and impairment in the neural functioning in higher concentrations (Rothweel and Strijbos 1995). Pro-inflammatory cytokines, and in particular IL-1 β , can modify all main classes of ion channels (voltage-gated Na⁺, Ca²⁺, K⁺ channels) and so they could change the neuronal excitability; they could be regarded not only as inflammatory mediators, but also as neuromodulators (Schäfers and Sorkin 2008). In fact, it has been demonstrated that highly purified neurons in vitro (Vezzani and Viviani 2015) and sensory neurons (Miller et al. 2009) do express cytokine receptors and action potentials can be initiated after cytokine levels' changes (Miller et al. 2009). The activation of cytokine receptors in neurons rapidly and persistently alters their excitability, in particular, specific interactions have been reported between IL-1 β , TNF- α , IL-6 and glutamatergic or GABAergic neurotransmission. An abnormal

activation of neurons, following cytokine signals modulating their excitability, could elicit excitotoxicity (Vezzani and Viviani 2015). We observed neurological deficits prevalently in the sensory function and reflexes. We can speculate that alterations in the neurons' excitability could explain the prevalence of these kinds of deficits evidenced by the neurological examination.

Conclusion

Our results suggest that IL-1 β -511 SNP could be a marker of susceptibility to chemotherapy-induced neurotoxicity.

Moreover, IL-1 β circulating levels, possibly associated to T allele at -511 position in IL-1 β SNP, could promote neuro-inflammation processes leading to neurodegeneration. The activation of molecular/cellular pathways that lead to neurodegeneration is less probable in patients with SNPs associated to lower levels of cytokines, for example, in CC homozygosity in the IL-1 β gene -511 C>T polymorphism.

In the context of cancer treatment, we suggested the potential value of IL-1 β -511 C>T as genetic biomarkers to identify patients with higher risk to develop neurological deficits and possible targets in the development of more effective treatments.

Limits

This study should be considered a preliminary/exploratory work, and results need to be confirmed in larger groups, using quantitative technique to estimate the sample size.

The heterogeneity of therapies and pathologies included in our sample could allow exploring shared pathophysiological mechanisms, but could also blur other effects.

Therefore, studies with more homogeneous populations and longitudinal evaluations of chemotherapy-induced changes should be performed.

References

- Ahles TA, Saykin AJ, Furstenberg CT, et al (2002) Neuropsychologic impact of standard-dose systemic chemotherapy in long-term survivors of breast cancer and lymphoma. *J Clin Oncol* 20:485–493. doi: 10.1200/JCO.20.2.485
- Arend WP (2002) The balance between IL-1 and IL-1Ra in disease. *Cytokine Growth Factor Rev* 13:323–340.
- Argyriou AA, Bruna J, Marmiroli P, Cavaletti G (2012) Chemotherapy-induced peripheral neurotoxicity (CIPN): An update. *Crit Rev Oncol Hematol* 82:51–77.
- Argyriou AA, Iconomou G, Kalofonos HP (2008a) Bortezomib-induced peripheral neuropathy in multiple myeloma: A comprehensive review of the literature. *Blood* 112:1593–1599.
- Argyriou AA, Polychronopoulos P, Iconomou G, et al (2008b) A review on oxaliplatin-induced peripheral nerve damage. *Cancer Treat Rev* 34:368–377.
- Baudino B, D'agata F, Caroppo P, et al (2012) The chemotherapy long-term effect on cognitive functions and brain metabolism in lymphoma patients. *Q J Nucl Med Mol Imaging* 56:559–568.
- Di Bona D, Plaia A, Vasto S, et al. (2008) Association between the interleukin-1beta polymorphisms and Alzheimer's disease: a systematic review and meta-analysis. *Brain Res Rev* 59:155–163. doi: 10.1016/j.brainresrev.2008.07.003
- Bower JE, Ganz PA, Irwin MR, et al (2013) Cytokine genetic variations and fatigue among patients with breast cancer. *J Clin Oncol* 31:1656–1661. doi: 10.1200/JCO.2012.46.2143
- Cavaletti G, Alberti P, Marmiroli P (2011) Chemotherapy-induced peripheral neurotoxicity in the era of pharmacogenomics. *Lancet Oncol* 12:1151–1161.

- Cavaletti G, Cavaletti E, Oggioni N, et al (2000) Distribution of paclitaxel within the nervous system of the rat after repeated intravenous administration. *Neurotoxicology* 21:389–393.
- Cavaletti G, Marmiroli P (2015) Chemotherapy-induced peripheral neurotoxicity. *Curr Opin Neurol* 28:500–507. doi: 10.1097/WCO.0000000000000234
- Cavaletti G, Marmiroli P (2010) Chemotherapy-induced peripheral neurotoxicity. *Nat Rev Neurol* 6:657–666. doi: 10.1517/14740338.3.6.535
- Cavallone L, Bonafè M, Olivieri F, et al (2003) The role of IL-1 gene cluster in longevity: A study in Italian population. *Mech Ageing Dev* 124:533–538.
- Cleeland CS, Bennett GJ, Dantzer R, et al (2003) Are the symptoms of cancer and cancer treatment due to a shared biologic mechanism? A cytokine-immunologic model of cancer symptoms. *Cancer* 97:2919–2925. doi: 10.1002/cncr.11382
- del Rey A, Apkarian AV, Martina M, Besedovsky HO (2012) Chronic neuropathic pain-like behavior and brain-borne IL-1 β . *Ann N Y Acad Sci* 1262:101–107. doi: 10.1111/j.1749-6632.2012.06621.x
- Déniz-Naranjo MC, Muñoz-Fernandez C, Alemany-Rodríguez MJ, et al (2008) Cytokine IL-1 beta but not IL-1 alpha promoter polymorphism is associated with Alzheimer disease in a population from the Canary Islands, Spain. *Eur J Neurol* 15:1080–1084. doi: 10.1111/j.1468-1331.2008.02252.x
- Dinarello CA (1994) The biological properties of interleukin-1. *Eur Cytokine Netw* 5:517–531.
- El-Omar EM, Carrington M, Chow WH, et al (2000) Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 404:398–402. doi: 10.1038/35006081

- Forlenza OV, Diniz BS, Talib LL, et al (2009) Increased serum IL-1beta level in Alzheimer's disease and mild cognitive impairment. *Dement Geriatr Cogn Disord* 28:507–512. doi: 10.1159/000255051
- Ganz PA, Bower JE, Kwan L, et al (2013) Does tumor necrosis factor-alpha (TNF- α) play a role in post-chemotherapy cerebral dysfunction? *Brain Behav Immun* 30 Suppl:S99–108. doi: 10.1016/j.bbi.2012.07.015
- di Giovine FS, Takhsh E, Blakemore AI, Duff GW (1992) Single base polymorphism at -511 in the human interleukin-1 beta gene (IL1 beta). *Hum Mol Genet* 1:450. doi: 10.1093/hmg/1.6.450
- Green EK, Harris JM, Lemmon H, et al (2002) Are interleukin-1 gene polymorphisms risk factors or disease modifiers in AD? *Neurology* 58:1566–1568. doi: 10.1212/WNL.58.10.1566
- Griffin WS (2006) Inflammation and neurodegenerative diseases. *Am Clin Nutr* 83:470S–474S.
- Grimaldi LM, Casadei VM, Ferri C, et al (2000) Association of early-onset Alzheimer's disease with an interleukin-1alpha gene polymorphism. *Ann Neurol* 47:361–365.
- Hall SK, Perregaux DG, Gabel CA, et al (2004) Correlation of polymorphic variation in the promoter region of the interleukin-1 β gene with secretion of interleukin-1 β protein. *Arthritis Rheum* 50:1976–1983. doi: 10.1002/art.20310
- Hausheer FH, Schilsky RL, Bain S, et al (2006) Diagnosis, management, and evaluation of chemotherapy-induced peripheral neuropathy. *Semin Oncol* 33:15–49.
- Henderson ST, Poirier J (2011) Pharmacogenetic analysis of the effects of polymorphisms in APOE, IDE and IL1B on a ketone body based therapeutic on cognition in mild to moderate Alzheimer's disease; a randomized, double-blind, placebo-controlled study. *BMC Med Genet* 12:137.

- Huang D, Pirskanen R, Hjelmström P, Lefvert AK (1998) Polymorphisms in IL-1beta and IL-1 receptor antagonist genes are associated with myasthenia gravis. *J Neuroimmunol* 81:76–81.
- Huang D, Zheng C, Giscombe R, et al (1999) Polymorphisms at -174 and in the 3' flanking region of interleukin-6 (IL-6) gene in patients with myasthenia gravis. *J Neuroimmunol* 101:197–200. doi: 10.1016/S0165-5728(99)00140-X
- Inagaki M, Yoshikawa E, Matsuoka Y, et al (2007) Smaller regional volumes of brain gray and white matter demonstrated in breast cancer survivors exposed to adjuvant chemotherapy. *Cancer* 109:146–156. doi: 10.1002/cncr.22368
- Licastro F, Pedrini S, Ferri C, et al (2000) Gene polymorphism affecting alpha1-antichymotrypsin and interleukin-1 plasma levels increases Alzheimer's disease risk. *Ann Neurol* 48:388–391.
- Miller BJ, Buckley P, Seabolt W, et al (2011) Meta-analysis of cytokine alterations in schizophrenia: Clinical status and antipsychotic effects. *Biol Psychiatry* 70:663–671. doi: 10.1016/j.biopsych.2011.04.013
- Miller RJ, Jung H, Bhangoo SK, White FA (2009) Cytokine and chemokine regulation of sensory neuron function. *Handb Exp Pharmacol.* 194:417–449. doi: 10.1007/978-3-540-79090-7_12
- Natori A, Ogata T, Sumitani M, et al (2015) Potential role of pNF-H, a biomarker of axonal damage in the central nervous system, as a predictive marker of chemotherapy-induced cognitive impairment. *Clin Cancer Res* 21:1348–52. doi: 10.1158/1078-0432.CCR-14-2775
- Nicklin MJ, Weith A, Duff GW (1994) A physical map of the region encompassing the human interleukin-1 alpha, interleukin-1 beta, and interleukin-1 receptor antagonist genes. *Genomics* 19:382–384.

O'Farrell E, MacKenzie J, Collins B (2013) Clearing the air: A review of our current understanding of "chemo fog." *Curr Oncol Rep* 15:260–269. doi: 10.1007/s11912-013-0307-7

Pettersson CA V, Sharma HS, Olsson Y (1990) Vascular permeability of spinal nerve roots. A study in the rat with Evans blue and lanthanum as tracers. *Acta Neuropathol* 81:148–154. doi: 10.1007/BF00334503

Pociot F, Mølvig J, Wogensen L, et al (1992) A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro. *Eur J Clin Invest* 22:396–402.

Poli F, Nocco A, Berra S, et al (2002) Allele frequencies of polymorphisms of TNFA, IL-6, IL-10 and IFNG in an Italian Caucasian population. *Eur J Immunogenet* 29:237–240. doi: 10.1046/j.1365-2370.2002.00303.x

Ren K, Torres R (2009) Role of interleukin-1beta during pain and inflammation. *Brain Res Rev* 60:57–64.

Rothwell NJ, Strijbos PJ (1995) Cytokines in neurodegeneration and repair. *Int J Dev Neurosci* 13:179–185.

del Rey A, Apkarian AV, Martina M, Besedovsky HO (2012) Chronic neuropathic pain-like behavior and brain-borne IL-1 β . *Ann N Y Acad Sci* 1262:101–107. doi: 10.1111/j.1749-6632.2012.06621.x

Santtila S, Savinainen K, Hurme M (1998) Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production in vitro. *Scand J Immunol* 47:195–198. doi: 10.1046/j.1365-3083.1998.00300.x

Schäfers M, Sorkin L (2008) Effect of cytokines on neuronal excitability. *Neurosci Lett* 437:188–193. doi: 10.1016/j.neulet.2008.03.052

- Seretny M, Currie GL, Sena ES, et al (2014) Incidence, prevalence, and predictors of chemotherapy-induced peripheral neuropathy: A systematic review and meta-analysis. *Pain* 155:2461–2470. doi: 10.1016/j.pain.2014.09.020
- Seripa D, Dobrina A, Margaglione M, et al (2003) Relevance of interleukin-1 receptor antagonist intron-2 polymorphism in ischemic stroke. *Cerebrovasc Dis* 15:276–281. doi: 10.1159/000069497
- Shafia S, Dilafroze, Sofi FA, et al (2014) Rheumatoid arthritis and genetic variations in cytokine genes: a population-based study in Kashmir Valley. *Immunol Invest* 43:349–59. doi: 10.3109/08820139.2013.879171
- Skundric DS, Dai R, James J, Lisak RP (2002) Activation of IL-1 signaling pathway in Schwann cells during diabetic neuropathy. *Ann N Y Acad Sci* 958:393–398.
- Smith EML, Beck SL, Cohen J (2008) The total neuropathy score: a tool for measuring chemotherapy-induced peripheral neuropathy. *Oncol Nurs Forum* 35:96–102. doi: 10.1188/08.ONF.96-102
- Smith JA, Das A, Ray SK, Banik NL (2012) Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases. *Brain Res Bull* 87:10–20. doi: 10.1016/j.brainresbull.2011.10.004
- Sommer C, Kress M (2004) Recent findings on how proinflammatory cytokines cause pain: Peripheral mechanisms in inflammatory and neuropathic hyperalgesia. *Neurosci Lett* 361:184–187.
- Spinnler H, Tognoni G (1987) Standardizzazione e taratura italiana di test neuropsicologici.
- Stefanidis I, Kreuer K, Dardiotis E, et al (2014) Association Between the Interleukin-1 β Gene (IL1B) C-511T Polymorphism and the Risk of Diabetic Nephropathy in Type 2

- Diabetes: A Candidate-Gene Association Study. *DNA Cell Biol* 33:463–8. doi: 10.1089/dna.2013.2204
- Vezzani A, Viviani B (2015) Neuromodulatory properties of inflammatory cytokines and their impact on neuronal excitability. *Neuropharmacology* 96:70–82. doi: 10.1016/j.neuropharm.2014.10.027
- Vichaya EG, Chiu GS, Krukowski K, et al (2015) Mechanisms of chemotherapy-induced behavioral toxicities. *Front Neurosci* 9:131. doi: 10.3389/fnins.2015.00131
- Wang XM, Lehky TJ, Brell JM, Dorsey SG (2012) Discovering cytokines as targets for chemotherapy-induced painful peripheral neuropathy. *Cytokine* 59:3–9.
- Wang X-M, Walitt B, Saligan L, et al (2015) Chemobrain: a critical review and causal hypothesis of link between cytokines and epigenetic reprogramming associated with chemotherapy. *Cytokine* 72:86–96. doi: 10.1016/j.cyto.2014.12.006
- Weisman D, Hakimian E, Ho GJ (2006) Interleukins, Inflammation, and Mechanisms of Alzheimer's Disease. *Vitam Horm* 74:505–530.
- Wilkes G (2007) Peripheral Neuropathy Related to Chemotherapy. *Semin Oncol Nurs* 23:162–173. doi: 10.1016/j.soncn.2007.05.001
- Wilson AG, di Giovine FS, Blakemore AI, Duff GW (1992) Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. *Hum Mol Genet* 1:353. doi: 10.1093/hmg/1.5.353
- Windebank AJ, Grisold W (2008) Chemotherapy-induced neuropathy. *J Peripher Nerv Syst* 13:27–46. doi: 10.1111/j.1529-8027.2008.00156.x

Table 1: Primers and conditions of PCR and digestion pattern

| Polymorphism | Primers | Ta | Product | Restriction Enzymes | Fragment expected |
|---------------------------|--|------|---------|-----------------------|---------------------------------|
| IL-1 α -889 C>T | F: AAGCTTGTCTACCA CCTGAACTAGGC R: TTACATATGAGCCTT CCATG | 54°C | 99 bp | NcoI (Fermentas) | C=83+16 bp T=99 bp |
| IL-1 β -511 C>T | F: TGGCATTGATCTGGTT CATC R: GTTTAGGAATCTCCC ACTT | 54°C | 304 bp | Eco881 (Fermentas) | C=190+114 bp T=304 bp |

Table 2: Demographic and clinical data

| Variables | CHT | No-CHT | p |
|--------------------------------|--------------|--------------|------------------|
| Number (M/F) | 49 (24M/25F) | 36 (22M/14F) | .282 |
| Age [y] | 49 ± 14 | 58 ± 13 | .003 |
| Education [y] | 12 ± 4 | 11 ± 4 | .247 |
| Smoke | 12% | 14% | .734 |
| BMI [kg/m^2] | 25 ± 4 | 24 ± 6 | .677 |
| Histotype H/S% | 73/27% | 67/33% | .619 |
| Disease free% | 71% | 0% | < .001 |
| Time from CHT [mos] | 15 ± 23 | - | - |
| Cycles Number | 6 ± 4 | - | - |
| WBC [$10^9/\text{L}$] | 6.5 ± 4.0 | 7.3 ± 2.5 | .363 |
| RBC [$10^{12}/\text{L}$] | 4.4 ± 0.8 | 4.8 ± 0.6 | .006 |
| HbG [g/dL] | 13.1 ± 4.6 | 14.3 ± 1.8 | .187 |
| Glucose [mg/dL] | 101.4 ± 17.9 | 102.3 ± 15.5 | .810 |
| Cortisol [nmol/L] | 133.9 ± 59.8 | 145.6 ± 55.9 | .447 |

BMI = Body Mass Index, H = Hematologic, S = Solid, WBC = White Blood Cells, RBC = Red Blood Cells, HbG = Glycated Haemoglobin, CHT = chemotherapy treated cancer patients, No-CHT = not treated cancer patients, p = p value comparing CHT and No-CHT groups

Table 3: Neuropsychological and psychological data

| Variables | CHT | No-CHT | p |
|-----------------------|------------|------------|------|
| | | | |
| Neurological Deficits | 59% | 21% | .004 |
| MMSE | 27.6 ± 2.0 | 28.3 ± 1.2 | .044 |
| Phonemic Fluency | 31 ± 10 | 35 ± 9 | .041 |
| SST | 10 ± 3 | 10 ± 3 | .828 |
| TMT B [s] | 93 ± 51 | 82 ± 46 | .343 |
| | | | |
| HADS-D | 5 ± 4 | 6 ± 4 | .358 |
| HADS-A | 6 ± 4 | 7 ± 4 | .249 |
| DT | 4 ± 3 | 4 ± 3 | .847 |

MMSE = Mini Mental State Examination, SST = Short Story Test, TMT = Trail Making Test, HADS = Hospital Anxiety and Depression Scale, DT = Distress Thermometer, CHT = chemotherapy treated cancer patients, No-CHT = not treated cancer patients, p = p value comparing CHT and No-CHT groups

Table 4: Cancers and chemotherapy distribution

| Variables | CHT | No-CHT | Total |
|-------------------------|------------|---------------|--------------|
| Hodgkin's lymphomas | 11 (31%) | 8 (33%) | 19 (32%) |
| Non-Hodgkin's lymphomas | 25 (69%) | 16 (67%) | 41 (68%) |
| ABVD | 8 (73%) | - | - |
| CHOP | 17 (68%) | - | - |
| Rituximab | 20 (56%) | - | - |
| Breast cancer | 8 (62%) | 2 (17%) | 10 (40%) |
| Lung cancer | 2 (15%) | 3 (25%) | 5 (20%) |
| Duodenal-rectal cancer | 1 (8%) | 4 (33%) | 5 (20%) |
| Other | 2 (15%) | 3 (25%) | 5 (20%) |
| Epirubicin | 7 (54%) | - | - |
| Fluorouracil | 6 (46%) | - | - |
| Cyclophosphamide | 4 (31%) | - | - |
| Taxanes | 4 (31%) | - | - |
| Platinum-based | 2 (15%) | - | - |

CHT = chemotherapy treated cancer patients, No-CHT = not treated cancer patients,

ABVD = Adriamycin/Hydroxydaunorubicin/Doxorubicin, Bleomycin, Vinblastine and Dacarbazine,

CHOP = Cyclophosphamide, Hydroxydaunorubicin, Oncovin/Vincristine and Prednisone or Prednisolone

Table 5: Genotype distributions of SNPs in CHT and No-CHT patients

| IL-1β | Controls | CHT | p^a | p^b | No-CHT | p^a | p^b | p^c |
|--------------------------------|-----------------|------------|----------------------|----------------------|---------------|----------------------|----------------------|----------------------|
| CC | 41% | 39% | .962 | .979 | 53% | .375 | .208 | .442 |
| CT | 45% | 47% | | | 36% | | | |
| TT | 14% | 14% | | | 11% | | | |
| IL-1α | Controls | CHT | p^a | p^b | No-CHT | p^a | p^b | p^c |
| CC | 49% | 49% | .777 | .117 | 53% | .395 | .360 | .484 |
| CT | 42% | 37% | | | 42% | | | |
| TT | 9% | 14% | | | 5% | | | |

Controls data were from literature (Poli et al. 2002; Cavallone et al. 2003; Seripa et al. 2003), CHT = chemotherapy treated cancer patients, No-CHT = not treated cancer patients, p^a = p value comparing distributions with HC, p^b = p value controlling for Hardy-Weinberg equilibrium, p^c = p value comparing CHT and No-CHT distributions

Table 6: IL-1 β -511 genotype stratification in CHT and No-CHT patients

| | CHT | | | No-CHT | | |
|------------------------------|-------------|-------------|------|------------|------------|------|
| Genotype | CT/TT | CC | p | CT/TT | CC | p |
| Patients | 61% | 39% | - | 47% | 53% | - |
| Neurological deficits | 73% | 37% | .029 | 25% | 17% | .999 |
| Time from CHT | 18 ± 31 mos | 13 ± 16 mos | .831 | - | - | - |
| CHT Cycles | 6 ± 5 | 7 ± 3 | .421 | - | - | - |
| Age | 49 ± 12 y | 49 ± 16 y | .983 | 59 ± 15 | 57 ± 12 | .709 |
| Education | 12 ± 3 y | 12 ± 4 y | .918 | 12 ± 4 | 11 ± 4 | .363 |
| MMSE | 27.2 ± 2.5 | 27.9 ± 1.7 | .238 | 28.5 ± 1.3 | 28.2 ± 1.1 | .430 |
| HADS-D | 5 ± 4 | 5 ± 4 | .868 | 6 ± 5 | 6 ± 4 | .654 |

CHT = chemotherapy treated cancer patients, No-CHT = not treated cancer patients, MMSE = Mini Mental State Examination, p = comparison between variables and genotypes in CHT and No-CHT groups

Table 7: Neurological examination deficits

| Variables | CHT | No-CHT | p |
|-------------------|-----|--------|-------------|
| Consciousness | 0% | 0% | - |
| Speech impairment | 0% | 0% | - |
| Motor function | 2% | 4% | .999 |
| Sensory function | 41% | 19% | .057 |
| Balance and Gait | 2% | 0% | .999 |
| Reflexes | 57% | 17% | .002 |
| Cranial Nerves | 2% | 0% | .999 |

CHT = chemotherapy treated cancer patients, No-CHT = not treated cancer patients,
 p = p value comparing groups

Table 8: Neurological examination deficits in the CHT subgroup

| Variables | CT/TT | CC | p |
|-------------------|-------|-----|------|
| Consciousness | 0% | 0% | - |
| Speech impairment | 0% | 0% | - |
| Motor function | 4% | 0% | .999 |
| Sensory function | 46% | 31% | .518 |
| Balance and Gait | 4% | 0% | .999 |
| Reflexes | 65% | 44% | .210 |
| Cranial Nerves | 4% | 0% | .999 |

CHT = chemotherapy treated cancer patients, No-CHT = not treated cancer patients,
p = p value comparing groups