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"Exploring the Relationship between Ageing and Cancer: from Translational to Clinical Research"

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SUMMARY OF THE RESEARCH ACTIVITY

EXPLORING THE RELATIONSHIP BETWEEN AGEING AND CANCER: FROM TRANSLATIONAL TO CLINICAL RESEARCH.

INTRODUCTION

A better understanding of the physiological and functional changes that occur with aging will enable to improve strategies for treating elderly cancer patients. For istance, hemathological toxicity is a major obstacle to the administration of chemotherapy in elderly cancer patients and ageing per sé is a major risk factor for cancer development, but the age-related impairment of immune system has never been studied in cancer patients.

For this reason, the present Doctoral Course has been committed to provide the first description of immune senescence observed in cancer patients. In the context of a prospective, exploratory study, TREC levels, subsets of peripheral naïve/memory T-cells and peripheral cell telomere length have been detected in elderly cancer patients and in age-matched controls.

A further critical issue of geriatric oncology is to uncover clinical problems that may impair the potential benefits and tolerability of anticancer treatments (Balducci, 2003; Extermann, 2003).

Recently, the International Society of Geriatric Oncology released a position paper where the obligatory integration of a comparable form of geriatric assessment is strongly recommended in future studies (Wildiers, 2013). The Multidimensional Prognostic Index (MPI) has been developed from a complete CGA and, differently from it, MPI may be administered and scored in a consistent manner. In order to answer this prioritary issue of geriatric oncology, the second project of this doctoral research program has been devoted to validate the MPI in patients with advanced cancer (Pilotto, 2008) to predict the 6 and 12-months overall mortality risk. In addition to estimate the tumor-independent survival, a CGA is essential when planning a cancer treatment as it uncovers medical conditions that may worsen the chemotherapy toxicity reported in clinical trials involving younger patients (Balducci, 2007). Despite there is strong evidence that any treatment decision in elderly cancer patients should be supported by a CGA, this is still performed in less than 10% of cancer centers because it is highly time-consuming. For this reason various author attempted to summarize the complete CGA in shorter versions. Among these screening tests, the Vulnerable Elders Survey (VES-13), a simple 13-item questionnaire has good sensitivity and acceptable

specificity (Luciani, 2010) in comparison with a full CGA, but there is not consistent medical literature regarding its ability to predict chemotherapy toxicity. Therefore, the third chapter of the present Doctoral Research Program reports a joint analysis of 4 prospective studies that evaluated the accuracy of VES-13 in predicting the risk of high grade toxicity in elderly patients undergoing chemotherapy.

PATIENTS AND METHODS.

Immunesenescence and Cancer. Fifty-two elderly patients with breast or colorectal cancer and 39 age-matched controls without personal history of cancer were enrolled. All patients underwent a Comprehensive Geriatric Assessment (CGA), from which a multidimensional prognostic index (MPI) score was calculated. Peripheral blood samples were collected at the time of enrollment, prior to any oncological medical treatment (endocrine therapy, chemo- therapy, radiotherapy or immune therapy). Peripheral blood samples were studied for naïve and recent thymic emigrant (RTE) $CD4^+$ and $C8^+$ cells by flow cytometry. T-cell receptor rearrangement excision circle (TREC) levels, telomere length and telomerase activity in peripheral blood cells were quantified by real-time PCR. In addition to descriptive analysis through Mann–Whitney U test and Student's t-test, correlations between age and TREC levels, or telomere length in both groups were analyzed with Pearson's $\chi 2$ test. TREC levels and telomere length were also analyzed as dichotomous variables (cut-off: ≤median) and Odd Ratios were estimated with a logistic regression model.

Validation of MPI in Cancer Patients. Patients aged 70 yrs and older with a recently-diagnosed metastatic or inoperable cancer were enrolled and received a complete CGA including functional state, comorbidity, cognitive and humoral state, nutritional state, risk of pressure scores, social aspect and medications. The MPI score was calculated for each patient from the results of the various tests (ADL, IADL, SPMSQ, CIRS-CI, MNA, ESS, number of drugs, and social conditions), as reported elsewhere by Pilotto et al (Pilotto, 2007).

Statistical Analysis. The associations between 6- or 12-months mortality and the MPI scores, was analyzed using a Cox's proportional hazards regression model adjusted for age and gender. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated to estimate the strength of the associations. The discriminatory power of the mortality model at 6 and 12 months of follow-up was assessed by calculating the area under the ROC curves for the MPI (considered as a continuous variable) using logistic regression models.

VES-13 to predict chemotherapy toxicity. The study involved patients aged >70 years with a diagnosis of a solid or hematological tumor. All Patients were administered VES-13. For all patients number of medications, comorbidities, CIRS-G score and index, type of chemotherapy, line of treatment, MMSE and MNA scores were recorded. Grade 3-4 hematological and non hematological toxicities were avaibile for all patients. Regression analysis was performed.

RESULTS

Immunesenescence and Cancer. The percentages of CD8+ naïve and CD8+ RTE cells and TREC levels were significantly lower in cancer patients than in controls ($p = 0.003$, $p = 0.004$, $p = 0.031$, respectively). Telomere lengths in peripheral blood cells were significantly shorter in cancer patients than in controls ($p = 0.046$) and did not correlate with age in patients, whereas it did in controls ($r = -0.354$, $p = 0.031$). Short telomere (≤median)/low TREC (≤median) profile was associated with higher risk of cancer (OR = 3.68 [95% CI 1.22–11.11]; $p = 0.021$). Neither unfitness on CGA nor MPI score were significantly related to thymic output or telomere length in either group.

Validation of MPI in Cancer Patients. A hundred and sixty patients entered the study. The MPI-related hazard ratios were higher at 6 months of follow-up than at 12 months, a high MPI being associated with a HR of 8.094 (95% CI 3.749-17.475, $p<0.0001$) at 6 months as opposed to 5.655 (95% CI 2.866-11.158, $p<0.0001$) at 12 months. When the MPI was considered as a continuous variable, any increase by 0.2 units (corresponding to the lower quartile) was associated with a

2.347-fold increase in the mortality risk (95% CI=1.838-2.997) at 6 months and a 2.051-fold increase (95% CI=1.662-2.531) at 12 months. The discriminatory power of the MPI's predictive performance was statistically significant.

The age- and sex-adjusted area under the ROC curve for MPI score at 6 and 12 months of follow-up were 0.81 (95% CI, 0.74-0.88) and 0.78 (95% CI, 0.71-0.85), respectively.

VES-13 to predict chemotherapy toxicity. 648 patients aged ≥ 66 years old were included, mean age was 76.2 years (SD 4.5, 66 to 90), 336 (51.9%) were female. VES-13 identified 287 of the patients (44.3%) as vulnerable. Grade 3-4 hematological and non-hematological toxicities were more prevalent in the vulnerable subjects (35.2% vs 20.8%, p <0.0001, and 18.5% vs 10.8%, p = 0.0055). Vulnerable patients (OR) had a higher risk of hematological and non hematological toxicity with an OR 2.15, (95% CI 1.46-3.17; p<0.001) and 1.66 (95% CI 1.02-2.72; p = 0.043) respectively.

CONCLUSIONS

The study of Immunesenescence provided the first evidence that elderly cancer patients seemed to suffer from a more severe decline in thymic output and had a lower proportion of naïve $CD8^+$ cells than age-matched controls. In addition, cancer patients had significantly shorter telomeres in their peripheral blood cells than age-matched non-cancer patients. This result suggests the unpublished hypothesis – which would need to be tested in a larger study - that elderly people with shorter telomeres are at higher risk of developing cancer. If confirmed, thymic output and telomere length could be widely used in elderly general population to easily identify subjects who run an higher risk of developing cancer and optimize the resources for screening procedures.

The second trial was the first to validate MPI in the oncological setting. The MPI retained in elderly patients with advanced cancer the same reliability and accuracy as reported in the original study by Pilotto et al. The results also suggested the possibility of creating a new, better-performing version of MPI by integrating it with the comorbidity severity index and the geriatric depression scale.

In the third study the patients identified as vulnerable by the VES-13 had a statistically significant higher risk of developing both hematological and non-hematological toxicity. These risk increases progressively with the aging of the population, particularly for haematological toxicity.

With the awareness that geriatric assessment of cancer patients cannot relies on a single test, future studies should be planned with the aim of prospectively identifying which is the most appropriate geriatric instrument for any single aspect of patient management (e.g. toxicity, overall survival, active life expectancy, or the quality of life) and clinical research.

SINTESI DELL'ATTIVITA' DI RICERCA SVOLTA

STUDIO DELLA RELAZIONE TRA CANCRO ED INVECCHIAMENTO ATTRAVERSO RICERCA TRANSLAZIONALE E CLINICA

INTRODUZIONE

Nonostante i casi di neoplasie solide nell'anziano siano in aumento, da decenni gli anziani sono sistematicamente esclusi dagli studi clinici in oncologia e questo implica una notevole difficoltà per gli oncologi a trasferire ai pazienti ultrasettantenni i risultati delle ricerche terapeutiche oncologiche.

Solo una migliore conoscenza delle modificazioni fisiologiche e funzionali che si accompagnano all'invecchiamento consentirebbe di migliorare le strategie di trattamento dei pazienti anziani con tumore. In particolare, nonostante la tossicità ematologica rappresenti il principale ostacolo alla somministrazione di chemioterapia e l'età avanzata sia di per sé un fattore di rischio per l'oncogenesi, non esiste in letteratura alcuno studio che abbia affrontato l'immunosenescenza nel paziente oncologico anziano. Perciò, parte considerevole dell'attività di ricerca svolta nel presente programma di Dottorato è stata dedicata ad uno studio prospettico di ricerca translazionale che ha confrontato l'output timico (livelli di T-cell receptor rearrangement excision circle – TREC- e subset di cellule T naïve e memoria) e la lunghezza dei telomeri in sangue periferico in una coorte di pazienti ultrasettantenni con diagnosi di neoplasia mammaria o colorettale con quelli riportati in anziani di pari età senza anamnesi personale di neoplasia.

Un'altra criticità dell'Oncologia Geriatrica è quella di standardizzare la valutazione geriatrica multidimensionale (CGA), strumento fondamentale per identificare possibili ostacoli all'efficacia ed alla tollerabilità dei trattamenti oncologici, per poterla utilizzare negli studi clinici.

Per rispondere a tale richiesta il secondo progetto di questo programma di Dottorato è stato finalizzato a validare per la prima volta nel setting oncologico il Multidimensional Prognostic Index (MPI), uno strumento codificato che deriva dalla CGA tradizionale. Nell'ambito di uno studio di Ricerca Ministeriale Finalizzata è stata valutata la capacità del MPI di predire la mortalità a 6 e 12 mesi in una coorte di pazienti anziani con neoplasia solida avanzata.

Terza priorità dell'oncologia geriatrica è di diffondere maggiormente l'uso della valutazione geriatrica. Attualmente infatti meno del 10% dei centri oncologici applica la CGA, che richiede

oltre 2 ore a paziente per la corretta esecuzione, mentre invece qualsiasi scelta terapeutica nel paziente anziano dovrebbe tenere conto di una CGA basale, anche allo scopo di identificare i pazienti a rischio di tossicità da chemioterapia. Per risolvere questa criticità negli ultimi anni sono stati introdotti dei test di screening fnializzati ad identificare i pazienti che meritano una valutazione geriatrica. Tra questi, la Vulnerable Elders Survey – 13 (VES-13) presenta una buona sensibilità e specificità rispetto alla CGA completa ma non era mai stata testata per predire la tossicità da chemioterapia. Pertanto, nel terzo studio riportato nella presente tesi sono stati analizzati i risultati di 4 studi prospettici che hanno valutato l'accuratezza della VES-13 nel predire il rischio di tossicità di alto grado in pazienti oncologici anziani in trattamento antiblatico.

PAZIENTI E METODI.

Immunesenescenza e Cancro. Cinquantadue pazienti con neoplasia mammaria o colorettale in stadio I-III e 39 controlli senza storia personale di tumore e di pari età sono stati arruolati nel presente studio e sottoposti inizialmente a CGA. Dopo la chirurgia e prima di iniziare qualsiasi trattamento medico adiuvante è stato prelevato sangue periferico per la determinazione citofluorimetrica di $CD4^+$ and $C8^+$ naïve e memoria e per la determinazione in real-time PCR dei livelli di TREC, della lunghezza dei telomeri nelle cellule periferiche e dell'attività telomerasica.

Validazione del MPI in pazienti oncologici. Pazienti ultrasettantenni e con recente diagnosi di neoplasia solida metastatica o inoperabile sono stati arruolati e sottoposti ad una CGA completa, comprendente lo stato funzionale, cognitive, umorale, le comorbidità, le medicazioni a domicilio, lo stato nutrizionale, il rischio di piaghe da decubito, gli aspetti sociali, da cui è stato calcolato il MPI come riportato da Pilotto et al (Pilotto, 2007). Per definire il valore prognostico di MPI sono stati utilizzati modelli di regressione Cox aggiustati per età e genere. Sono state inoltre calcolate le curve ROC attraverso modelli di regression logistica.

VES-13 e rischio di tossicità da chemioterapia. Quest'analisi combinata ha coinvolto pazienti ultrasettantenni con diagnosi di neoplasia solida o ematologica. Tutti i pazienti hanno compilato il

questionario VES-13. Sono stati riportati infine I dati relative al tipo di chemioterapia ricevuta, la linea di trattamento, la tossicità ematologica e non ematologica di grado 3-4 secondo I common toxicity criteria for adverse events (CTCAE). A questi dati è stata applicata un'analisi di regressione.

RISULTATI

Immunosenescenza e Cancro. La percentuale di cellule CD8+ naïve, CD8+ RTE ed i livelli di TREC sono risultati significativamente più bassi nei pazienti oncologici rispetto ai controlli ($p = 0.003$, $p = 0.004$, $p = 0.031$, rispettivamente). La lunghezza dei telomeri nelle cellule di sangue periferico era significativamente inferiore nei pazienti oncologici rispetto ai controlli ($p = 0.046$) e non correlava con l'età, come avveniva invece nei controlli ($r = -0.354$, $p = 0.031$). Il profilo con telomero corto (inferiori alla mediana) e bassi livelli di TREC (inferiori alla mediana) era significativamente associato con la diagnosi di neoplasia (OR = 3.68 [95% CI 1.22–11.11]; $p = 0.021$) mentre non vi era alcuna correlazione tra l'esito della CGA ed il punteggio MPI da un lato ed i marcatori di immunosenescenza dall'altro in entrambi i gruppi.

Validazione del MPI in pazienti oncologici. Lo studio ha coinvolto 160 pazienti. Gli hazard ratio correlati a MPI sfavorevole sono risultati significativamente più alti per la mortalità a 6 mesi rispetto a 12 mesi, più precisamente 8.094 (95% CI 3.749-17.475, $p<0.0001$) a 6 mesi e 5.655 (95% CI 2.866-11.158, $p<0.0001$) a 12 mesi. Quando MPI è stato valutato come una variabile continua, ogni incremento di 0.2 unità era associato ad un aumento di 2.347-volte del rischio di mortalità (95% CI=1.838-2.997) a 6 mesi e 2.051-volte (95% CI=1.662-2.531) a 12 mesi. Il valore prognostico di MPI è risultato statisticamente significativo. L'area delle curve ROC a 6 e 12 mesi, aggiustate per genere ed età era 0.81 (95% CI, 0.74-0.88) e 0.78 (95% CI, 0.71-0.85), rispettivamente.

VES-13 e rischio di tossicità da chemioterapia. Seicentoquarantotto pazienti di età ≥ 66 anni sono stati considerati nella presente analisi. Attraverso la VES-13 sono stati identificati 287 pazienti

vulnerabili. Gli eventi di tossicità ematologica e non ematologica di grado 3-4 sono risultati prevalenti nei soggetti vulnerabili (35.2% vs 20.8%, p <0.0001, e 18.5% vs 10.8%, p = 0.0055). Gli odd ratios per la tossicità ematologica e non ematologica nei pazienti vulnerabili sono risultati pari a 2.15, (95% CI 1.46-3.17; p<0.001) e 1.66 (95% CI 1.02-2.72; p = 0.043) rispettivamente.

CONCLUSIONI

Lo studio sull'immunosenescenza e cancro ha fornito la prima evidenza che i pazienti anziani oncologici, rispetto ai controlli sani, presentano una severa riduzione dell'output timico e della lunghezza dei telomeri in cellule di sangue periferico. Tali risultati suggeriscono l'ipotesi che i soggetti anziani con sistema immunitario senescente e telomeri più corti abbiano un rischio più alto di sviluppare neoplasie. Se tale associazione fosse confermata in una popolazione più ampia, i marcatori di immunosenescenza potrebbero essere impiegati per identificare gli anziani a rischio più elevato di neoplasia, sui quali concentrare le risorse per la diagnosi precoce.

Il secondo studio riportato è stato il primo a dimostrare che nei pazienti oncologici MPI mantiene il suo valore prognostico, con la stessa affidabilità ed accuratezza riportate nello studio originale di Pilotto et al. I risultati dello studio suggeriscono infine la possibilità di migliorare le prestazioni di MPI in oncologia integrandolo con l'indice di comorbidità e lo stato umorale.

Il terzo studio ha dimostrato che i pazienti giudicati vulnerabili alla VES-13 hanno un rischio significativamente più alto di sviluppare tossicità ematologica e non ematologica di alto grado. Tale rischio aumenta progressivamente con l'età, soprattutto per la tossicità eamtologica.

Con la consapevolezza che la valutazione geriatrica del paziente oncologico anziano non può essere esaustivamente svolta con un unico test, ulteriori studi dovranno essere condotti per identificare i test più appropriati per altri aspetti rilevanti per la gestione del paziente con neoplasia, ovvero la l'aspettativa di vita tumore-indipendente e la qualità di vita. Parallelamente vi è la necessità di studiare il processo di invecchiamento nell'anziano oncologico per identificare dei marcatori

molecolari di età biologica, che guidino in maniera oggettiva la scelta terapeutica in questa popolazione.

INTRODUCTION

EXPLORING THE RELATIONSHIP BETWEEN AGEING AND CANCER: FROM TRANSLATIONAL TO CLINICAL RESEARCH.

Persons over the age of 65 years are the fastest growing segment of the population and will account for an estimated 20% of Americans and 25% of Europeans by the year 2030 (Fries, 2003).

Cancer incidence is 11-fold higher in persons over the age of 65 years than in younger ones so that the number of incident cases of cancer in the elderly is expected to significantly increase in next decades (American Cancer Society, 2012).

Despite approximately 70% of deaths from cancer occur in patients aged 65 and over (Jemal A, 2010), the majority of clinical trial research in cancer care is conducted in younger patients (Hutchins, 1999). This discrepancy creates uncertainty for oncologists when extrapolating available data to treat their older patients. As result, elderly patients are less likely to be treated according to guidelines and their under-treatment may be detrimental to both survival and quality of life, as reported in several studies (Sargent, 2001; Bouchardy, 2003; Dale, 2003).

A better understanding of the physiological and functional changes that occur with aging will enable to improve strategies for treating elderly cancer patients. Since the aging process coincides with a gradual decline in the functional reserve of multiple organ systems (Balducci, 2003) the search for laboratory markers of biological aging and organ reserve should be a priority of research in the field of geriatric oncology.

Despite hemathological toxicity is a major obstacle to the administration of chemotherapy in elderly cancer patients, the age-related impairment of immune system has never been studied in cancer patients.

For this reason, the present Doctoral Course has been committed to provide the first description of immune senescence observed in cancer patients. In the context of a prospective, exploratory study, TREC levels, subsets of peripheral naïve/memory T-cells and peripheral cell telomere length have been detected in elderly cancer patients and in age-matched controls.

A further critical issue of geriatric oncology is to uncover clinical problems that may impair the potential benefits and tolerability of anticancer treatments (Balducci, 2003; Extermann, 2003). Really, the comprehensive geriatric assessment (CGA), a panel of clinical tools exploring physical,

humoral and cognitive impairment, concomitant diseases, medications and social state, is the only instrument available for oncologists to estimate tumour independent life-expectancy, uncover potential contraindications to antiblastic treatment and detect causes of additional risk of severe chemotherapy toxicity in elderly cancer patients (Extermann, 2005).

In the last decades, a large variety of CGAs was developed but none of them was chosen as reference, universally accepted, CGA model. Consequently, results by different studies cannot be compared and many outcomes that are of interest to older patients, such as functional impairment and independence, are not evaluated in traditional clinical trials (Falci, 2010).

Recently, the International Society of Geriatric Oncology released a position paper where the obligatory integration of a comparable form of geriatric assessment is strongly recommended in future studies (Wildiers, 2013). The Multidimensional Prognostic Index (MPI) has been recently developed from a complete CGA and, differently from it, MPI may be administered and scored in a consistent manner. In order to answer a prioritary issue of geriatric oncology, the lack of a largely accepted, standardized model of geriatric assessment, the second project of this doctoral research program has been devoted to validate the MPI in patients with advanced cancer (Pilotto, 2008).

In addition to estimate the tumor-independent survival, a CGA is essential when planning a cancer treatment as it uncovers medical conditions that may worsen the chemotherapy toxicity reported in clinical trials involving younger patients (Balducci, 2007). Despite there is strong evidence that any treatment decision in elderly cancer patients should be supported by a CGA, this is still performed in less than 10% of cancer centers because of the shortage of economic resources. In fact, CGA is highly time-consuming. For this reason various author attempted to summarize the complete CGA in shorter versions. These screening tools should distinghish fit patients, who deserve the same treatments of younger adults, from vulnerable patients, who need a full CGA (Overcash, 2004; Molina-Garrido, 2011; Hurria, 2011). Among these screening tests, the Vulnerable Elders Survey (VES-13), a simple 13-item function-based self-report questionnaire that has been developed and validated in a population of 6,000 community-dwelling U.S. Medicare beneficiaries aged 65 and

older, warrants the best performance to identify people at increased risk of death or functional decline (Saliba, 2001; Mohile, 2007).

In comparison with a full CGA, VES-13 has good sensitivity and acceptable specificity (Luciani, 2010), but there is not consistent medical literature regarding its ability to predict chemotherapy toxicity. Therefore, the third chapter of the present Doctoral Research Program reports the first prospective study that evaluated the accuracy of VES-13 in predicting the risk of high grade toxicity in elderly patients undergoing chemotherapy.

CHAPTER 1

The Cancer Risk

A Study of Immune Senescence in Elderly Cancer Patients

BACKGROUND AND AIMS

People over 65 years old are the fastest-growing age bracket in the population and will account for an estimated 20% of Americans and 25% of Europeans by the year 2030 (Fries, 2003). The incidence of malignancies increases with age, so the number of cancers in the elderly is expected to increase significantly in years to come (American Cancer Society, 2012). Data are becoming available that will enable a better use of chemotherapy in the older patient population (Hurria, 2012), but several studies have shown that elderly patients are less likely to be treated according to the guidelines, and their undertreatment can have a detrimental effect on both survival and quality of life (Bouchardy, 2003; Dale, 2003). Some studies have shown that elderly cancer patients may benefit from chemotherapy just as much as younger adults (Sargent, 2001; Muss, 2005), but at a higher risk of hematological toxicity (Muss, 2007). A better understanding of the physiological and functional changes that occur with aging will enable useful strategies for treating elderly cancer patients to be developed. Since the ageing process coincides with a gradual decline in the functional reserve of multiple organ systems (Balducci, 2003), the assessment of elderly cancer patients should not be based on their clinical features alone. The search for laboratory markers of biological aging and organ reserve should be a priority of clinical research in the field of geriatric oncology.

Over a lifetime, the immune system undergoes a profound remodeling process with a major impact on health and survival (Grubeck-Loebenstein, 2009; Fulop, 2010). Thymic involution and a diminished output of T lymphocytes are thought to be among the major factors contributing to the loss of immune function with age (Berzin, 1998). T cell output begins to decline exponentially from early on (Doeuk, 1998; Naylor, 2005) and by the a person reaches 75 years of age their immune repertoire appears to be severely impaired (Naylor, 2005). Recent data suggest, however, that the thymus may remain active even late in life, supplying functional T cells to the periphery (Nasi, 2006; Mitchell, 2010).

Measuring T cell receptor rearrangement excision circle (TREC) levels in peripheral blood lymphocytes has been suggested as a method for quantifying thymic output in different conditions (Douek, 1998; Zang, 1999; Ometto, 2002; De Rossi, 2002). TRECs are generated by T cell receptor gene rearrangement (Breit, 1997) and are maintained in thymic emigrant cells as DNA episomes. Because TRECs are not duplicated during mitosis, their concentration is diluted out with each cell division. The frequency of recent thymic emigrant (RTE) cells in peripheral blood, identified by the marker $CD31^+$ among the $CD45RA^+$ naïve T cells (Kimmig, 2002), decreases with aging and correlates well with the decline in TREC levels (Kohler, 2005; Junge, 2007). It has also been estimated, using a mathematical model describing human thymopoiesis, that the number of TREC-positive cells released every day from the thymus into the peripheral blood drops exponentially by 2 orders of magnitude during an 80-year lifespan (Ye, 2002).

Very little is known about the relationship between TRECs and cancer, especially in elderly patients. One study on patients with head and neck cancers, including just a few ≥70 years old, showed that the age-associated decrease in TREC-positive cells and naive $CD8^+$ and $CD4^+$ T cells was significantly greater in cancer patients than in controls, thus suggesting an altered lymphocyte homeostasis in cancer patients (Kuss, 2005).

Immune senescence affects the B-cell compartment too. A declining availability of T cell help and/or innate immune function could also contribute to changes in the B-cell compartment, interfering with B lymphopoiesis and homeostasis. These changes may ultimately exacerbate the decline in the protective qualities of antibodies produced by the elderly, increasing their sensitivity to infections and likelihood of developing cancer and some autoimmune syndromes (Sasaki, 2011). Whether aging leads to changes in serum immunoglobulin levels is still a matter of debate.

Immune system function depends largely on its capacity for extensive cell division and clonal lymphocyte expansion. Telomere length, and its regulation by telomerase have attracted considerable attention for their potential roles in controlling cell replication (Greider, 1998).

Telomeres are capping end structures of eukaryotic chromosomes essential for protecting chromosome integrity (Blackburn, 1991); they comprise a non-coding sequence of (TTAGGG)n repeats, in complex with the shelterin proteins (de Lange, 2009). Telomeres gradually become shorter with each cell division due to the inability of DNA polymerase to fully replicate the 3' ends of DNA. When a critical length is reached the cell undergoes cycle arrest and apoptosis (Blasco, 2005). Permanent cell growth relies on telomere maintenance and certain human cell subsets, as well as most cancer cells, have a telomerase activity that enables telomere *elongation* (Dolcetti, 2012). Despite their telomerase activity, most tumor cells have shorter telomeres than the corresponding normal tissues, and there is a relationship between short telomeres and genetic instability (Rampazzo, 2010; Garcia-Aranda, 2006).

Since telomere shortening reflects cell turnover and exposure to oxidative and inflammatory damage, which are crucial processes of biological aging, it has been suggested that telomere length may serve as an indicator of the aging process (Wong, 2003; Aviv 2006; Baird , 2006). Telomere shortening in peripheral blood cells has been associated with a number of chronic diseases, such as coronary heart disease, hypertension, dementia, obesity, insulin resistance, and osteoporosis. On the other hand, two clinical trials failed to confirm any relationship between telomere length and frailty syndrome in elderly non-cancer patients (Woo, 2008; Colerton, 2012).

Short telomeres in peripheral blood cells have been associated with a higher risk of head and neck, lung, breast, colorectal, bladder and renal cell cancer (Wu, 2003; Shao, 2007; Svenson, 2008). Telomere length in peripheral blood cells has also been independently associated with cancer-specific survival in patients with metastatic breast cancer, suggesting that this marker may be prognostic for overall survival in cancer patients generally (Svenson, 2008). To date, however, telomere length in peripheral blood cells has been measured in elderly non-cancer patients and in younger cancer patients, but not in elderly cancer patients.

Here we present the results of a prospective observational study that aimed to provide the first description of immune senescence markers (TREC levels, subsets of peripheral naïve and memory T-cells, interleukin (IL)-6 and immunoglobulin levels), telomere length and frailty scores in a sample of elderly cancer patients and age-matched controls.

1.2 PATIENTS AND METHODS

1.2.1 Study design and study population

This was a mono-institutional, exploratory study. Co-primary endpoints were to establish whether TREC levels and telomere lengths differ significantly between cancer patients and age-matched controls. The secondary endpoint was to assess the age effect on TREC levels and telomere lengths in both cancer patients and age-matched controls.

Patients aged ≥70 years diagnosed during the previous 2 months with stage I-III breast or colorectal cancer, and radically resected, who were consecutively admitted to the Oncology Division of the Veneto Institute of Oncology, were considered for enrolment in the study. Controls included patients ≥70 years old with no personal history of cancer, consecutively admitted to the Geriatric Clinic at Padova University. For both groups, the exclusion criteria were: any hematological disorders (also regarding the erythropoietic and myelocytic lines), chronic diseases requiring continuous immunosuppressive treatment (rheumatological disorders, autoimmune conditions, etc.), prior immunodeficiency, blood transfusion ≤4 weeks before blood sampling, active infectious diseases, extremely severe comorbidities suggesting a life expectancy <6 months, severe cognitive impairments hampering communication with the physician.

The study was approved by the Institutional Ethics Committee and conducted in accordance with the Helsinki Declaration and Good Clinical Practice guidelines. Written informed consent was obtained from all patients.

1.2.2 **Clinical assessment**

Complete demographic and clinical details were collected at the baseline for each patient (date of birth, gender, formal education, weight, height, body mass index (BMI), personal medical history, current medication; plus information, for cancer patients, about the primary tumor site, histotype, and stage, and any oncological treatment planned). Concomitantly with the first visit, both cases and controls underwent a traditional comprehensive geriatric assessment (CGA) administered by a multidisciplinary team that included a medical oncologist, a geriatrician and a psychologist. As reported elsewhere (Basso, 2004), the CGA included Activities of Daily Living (ADL) (Katz, 1970), Instrumental Activities of Daily Living (IADL) (Lawton, 1969), the Short Portable Mental Status Questionnaire (SPMSQ) (Pfeiffer, 1975) and the Mini Mental Status Examination (MMSE) (Folstein, 1975). Comorbidities and their severity were investigated using the Cumulative Illness Rating Scale-Comorbidity Index (CIRS-CI) and the Cumulative Illness Rating Scale-Severity Index (CIRS-SI) (Conwell, 1993). Affective status was assessed with the Geriatric Depression Scale (GDS) (Yesavage, 1983). Nutritional status was explored with the Mini Nutritional Assessment (MNA) (Guigoz et al, 1999). The risk of developing pressure sores was tested with the Exton Smith Scale (ESS) (Bliss, 1966). Social aspects included household composition, institutionalization, amount of assistance provided by a caregiver (average number of hours per day). The number of different drugs taken by patients for concomitant diseases was also recorded. The results of the CGA were interpreted as previously reported by Balducci (Balducci, 2003), classifying patients as fit, vulnerable or frail. To report the final outcome of the CGA in the form of a continuous variable, a multidimensional prognostic index (MPI) score was also calculated for each patient based on findings for the ADL, IADL, SPMSQ, CIRS-CI, CIRS-SI, MNA, ESS, number of drugs and social conditions, assuming a low mortality risk for MPI ≤ 0.33, a moderate risk for MPI between 0.34 and 0.66, and a high risk for MPI >0.66 (Pilotto, 2008).

1.2.3 Biomarker analyses

Peripheral blood samples were collected at the time of enrolment and analyzed for the standard blood parameters (listed in Table 1C), and for the following tests.

1.2.4 Flow cytometry

Peripheral blood mononuclear cells (PBMC) were isolated from peripheral blood by centrifugation on a Ficoll-Paque (Pharmacia, Uppsala, Sweden) gradient. Approximately 250 000 PBMC were stained for 15 min in the dark using the following labeled monoclonal antibodies (mAbs): anti-CD3 (fluorescein isothiocyanate [FITC]), anti-CD4 (peridinin chlorophyll protein [PerCP]), anti-CD8 (PerCP), anti-CD31 (phycoerythrin [PE]) and anti-CD45RA (allophycocyanin [APC]). Appropriate isotypic controls (mouse IgG1-PE and mouse IgG2b-APC) were used to assess non-specific staining. All mAbs were purchased from Becton-Dickinson (Becton-Dickinson Biosciences Pharmingen, San Diego, CA, USA). Cells were then washed with Automacs Buffer (Miltenyi Biotec Inc., Auburn, CA, USA) and resuspended in PBS supplemented with 1% paraformaldehyde. All samples were analyzed by four-color flow cytometry using a fluorescence-activated cell sorter (FACS) Calibur (Becton-Dickinson) equipped with a 488 nm argon-ion laser and a 635 nm red diode laser. A total of 50 000 events were collected in the lymphocyte gate using morphological parameters (forward- and side-scatter). Data were processed using CellQuest Pro Software (Becton-Dickinson) and analyzed using Kaluza® Analysis Software v.1.2 (Beckman Coulter, Inc.). The percentage of $CD45RA^+$ and $CD31^+$ cells was calculated within the $CD3^+CD4^+$ or $CD3^+CD8^+$ gate. $CD45RA^+$ cells were defined as naïve and $CD45RA^+CD31^+$ expression was defined as RTE (Kimming, 2002).

1.2.5 TREC quantification

Thymic output in PBMC was studied by measuring TREC levels by real-time polymerase chain reaction (PCR), exactly as described previously (Ometto, 2002; De Rossi, 2002). TREC levels were expressed as the number of TREC copies per 10^5 PBMC (De Rossi, 2002; Anselmi, 2007).

1.2.6 Telomere length measurement

Telomere length in PBMC was determined by real-time PCR exactly as described elsewhere (Rampazzo, 2010; Rampazzo, 2012) and values were expressed as the telomere to single copy gene (T/S) ratio (Rampazzo, 2010). In a set of experiments, telomere length was measured in $CD8^+$ cell subsets. $CD8^+$ T cells were isolated from PBMC using magnetic beads coated with mAb against human CD8 (CD8 MultiSort Kit, Miltenyi, Bergisch Gladbach, Germany). Then, using magnetic beads coated with CD45RO (CD45RO MicroBeads, Miltenyi), the $CD8^+$ cells were separated into $CD8^+CD45RO^+$ (memory cell subset) and $CD8^+CD45RO^-$ (naïve cell subset).

1.2.7 Telomerase activity quantification

Telomerase activity was quantified as explained elsewhere (Rampazzo, 2012). Briefly, one million cells were lysed in 20µL of CHAPS buffer and incubated at 4°C for 30 min. The lysate was then centrifuged at 12000g for 30 min at 4°C, and the supernatant was collected as reported previously (Trentin, 1999). Telomerase activity was assessed with a real-time PCR method, using 250 ng of cellular protein extract for each sample, exactly as described in (Rampazzo, 2012). Telomerase activity was expressed in relative units (RU).

1.2.8 Statistical analysis

Given the exploratory nature of the present study, a target sample size of 60 patients (30 patients per group) was calculated according to Browe (Browe, 1995). The enrolment target was increased

to 90 to ensure an adequate sample size should some of the blood samples be excluded due to laboratory-related issues.

Differences in geriatric parameters between cancer patients and controls were examined using the Mann-Whitney U test for non-parametric data and Pearson's χ^2 test with odds ratios (OR) and 95% confidence intervals (CI) for nominal data (CGA, MPI, ADL, IADL).

The Mann-Whitney U test was also used to compare subsets of lymphocytes, TREC levels and telomerase activity in cancer patients and controls. Correlations between age and TREC levels in both groups was analyzed with Pearson's test. Student's t-test was used to compare the telomere lengths detected in PBMC from cancer patients and controls, then the assumptions of normality and homogeneity were verified. The relationship between age and telomere length was assessed with Pearson's test in both groups. Spearman's rank correlation was used to analyze the associations between geriatric parameters and TREC levels, telomere lengths or telomerase activity.

All statistical analyses were performed using SPSS software, rel. 18 (SPSS Inc., Chicago, IL, USA). All *p*-values were two-tailed, and were considered significant when lower than 0.05.

1.3 RESULTS

1.3.1 Characteristics of the study population

Ninety-one patients, 52 with cancer (26 breast and 26 colorectal cancer) and 39 controls, were enrolled in the study. Their demographic and clinical characteristics are listed in Table 1A. There were more women in the control group (82.1%) than among the cancer patients (63.5%); the latter had better Karnofsky performance status than controls. As established by the study protocol, before enrollment, cancer patients underwent a thorough radiological assessment to rule out metastases. According to the TNM staging system, 16 patients (30.8%) had stage I, 33 (63.5%) stage II, and 3 (5.7%) stage III disease. Thirty- one patients were prescribed the same adjuvant therapy as for younger adults, according to good clinical practice, 18 patients received an adapted treatment, due

to problems detected at the CGA, and 3 were given no adjuvant therapy, due to frailty detected at the CGA and were only followed up.

1.3.2 Comprehensive geriatric assessment

All cancer patients and controls routinely underwent the full CGA (Table 1B). Concerning their social condition, only one cancer patient (1.9%) lived in a nursing home due to dependence in at least one ADL; all the others lived at home, 42 (80.8%) with their family, and 9 (17.3%) alone. All controls lived at home, 25 (64.1%) with their family, and 14 (35.9%) alone. Cancer patients and controls all benefited from the assistance of a caregiver for a median time of 24 hours a day (range 2-24 for cancer patients, 3-24 for controls).

When the CGA findings were interpreted as reported in (Balducci, 2003), 17.3% of the cancer patients and 43.6% of the controls were classified as *frail*, (OR=0.29, 95% CI 0.10-0.82; p=0.020). The proportion of *vulnerable* patients in the cancer (36.5%) and control (23.1%) groups did not differ significantly (OR=1.14, 95% CI 0.40-3.24, p=0.801) (Table 1B). The distribution of the MPI scores, grouped as low, moderate and severe (Pilotto, 2008), showed that 3 cancer patients (5.8%) and 9 controls (23.1%) had a moderate MPI (OR=0.20, 95% CI 0.05-0.79; p=0.022). The other 49 cancer patients (94.2%) and 29 controls (74.4%) had a low MPI, while only one control patient had a severe MPI score. When MPI was calculated as a continuous variable, the median score was significantly lower for cancer patients (0.19, range 0-0.44) than for controls (0.25, range 0.06-0.69), p=0.001 (Table 1B).

As for ADL and IADL, the cancer patients tended to have preserved a greater physical autonomy than the controls, though the difference was not statistically significant. The cancer patients' OR for independence in ADL and IADL was 2.44 (95% CI 0.88-6.74; p=0.790) and 0.11 (95% CI 0.81-4.57; p=0.133), respectively. The controls had a better cognitive status, in both the MMSE and SPMSQ, but their burden of their associated diseases (as shown by the comorbidity and severity indexes calculated with the CIRS) was higher, and so was their use of drugs for these comorbidities.

Finally, the two groups were fully comparable in terms of their nutritional and humoral status (Table 1B).

1.3.3 Standard hematological, biochemical parameters and CMV status

The hematological parameters of cancer patients and controls differed only in their platelet count (Table 1C). Controls had significantly lower levels of nutritional markers, such as total plasma proteins, triglycerides, and LDL-cholesterol; CRP and IL-6 were significantly higher in controls than in cancer patients (Table 1C). The overall prevalence of CMV-IgG seropositive individuals was 96.7%. Antibodies against CMV were found in 49 (94.2%) of cancer patients and 39 (100%) of controls. No statistical difference was found between CMV antibody titers between the groups ($p = 0.498$) (Table 1C).

1.3.4. Immune senescence markers

Cancer patients and controls had comparable percentages of total CD4+ lymphocytes, naïve CD4+ CD45RA+ CD27+ and RTE CD4+CD45RA+CD31+ cells (Table 1D). By contrast, the median (interquartile) percentages of naïve CD8+ CD45RA+ CD27+ and RTE CD8+CD45RA+CD31+ cells were significantly lower in cancer patients than in controls (16.7% [9.3–25.2] versus 24.6% [14.7–33.5]; $p = 0.003$) and (34.2% [24.7–46.4] versus 44.9% [36.0–50.7]; $p = 0.004$), respectively (Table 1D). Notably, the lower percentage of CD8+ naïve cells in cancer patients was compensated by a higher expansion of CD8+ memory cells; thus, cancer patients and controls did not differ in terms of their percentages of total CD8+ cells (Table 1D). In particular, among the CD8+ cell subsets, the CD45RA−CD27− (effector memory) cells were found to be significantly higher in cancer patients than in controls (21.7% [12.7–31.7] versus 16.3% [10.4–25.0]; $p = 0.042$). The imbalance in favor of the CD8 memory cell subset in cancer patients was evident even when the absolute cell count was considered (not shown). For a few subjects with available frozen samples (11 cancer patients and 8 controls), markers of immune senescence (CD28− CD57+) were

investigated. Cancer patients and controls exhibited similar percentages of CD4+CD28−CD57+, but CD8+ cells with senescent phenotype tended to be higher in the former (p = 0.082) (Table 1E).

TREC levels in PBMC were significantly higher in controls than in cancer patients (25.0 [14.0–56.0] versus 16.0 [7.7–31.5] TREC copies/10^5 PBMC; p=0.031) (Table 1D). TREC levels decreased significantly with increasing age in cancer patients (r = −0.478; p b 0.001), but did not correlate with age in controls (r = 0.071; p = 0.677) (Fig. 1A).

The mean telomere length in PBMC was significantly lower in cancer patients than in controls; the former had a mean (±standard deviation (SD)) telomere length of 1.09 ± 0.31 T/S versus 1.22 ± 0.30 T/S in controls, p=0.046 (Table 1D). Telomere length correlated inversely with age in controls (r = −0.354, p = 0.031), but not in cancer patients (r = −0.011, p = 0.938) (Fig. 1B). Telomerase activity was significantly higher in cancer patients, being detected in 38/52 patients (73.1%; median value 14.6 RU) and 20/38 controls (52.6%; median value 1.4 RU); p = 0.003. Telomerase activity was not influenced by age in either group.

TREC was defined as high or low, and telomere length as long or short according to their values above and below the median, respectively. Subjects with TREC low/telomere short profile were at higher risk of cancer than subjects with only low TREC or short telomere (Table 1E).

1.3.5. Relationship between TREC levels, telomere length and geriatric characteristics

In the control group, CIRS-SI was the only tool which revealed a significant positive association with TREC level (r = 0.45, p = 0.01) and telomere length (r = 0.35, p = 0.03), whereas it had a significant nega- tive correlation with the number of drugs taken for concomitant diseases (r = −0.39, p = 0.02) (Table 1F). No relationship emerged between telomere length, thymic output and geriatric features.

of cancer patients (Table 1F). Neither a classification of unfitness at CGA (not shown) nor the MPI score (Table 1F) correlated significantly with thymic output or telomere length in either group. Among the controls, TREC levels correlated positively with IL-6 (r = 0.34, p = 0.04) and negatively

with total plasma protein levels ($r = -0.38$, $p = 0.02$). Neither of these correlations emerged in the cancer patients (data not shown).

1.4 DISCUSSION

This is the first study aiming to shed light on two essential aspects of the ageing process (thymic output and telomere length in peripheral blood cells) in elderly cancer patients. These markers were studied in a sample of breast or colorectal cancer patients aged ≥70 years, as compared with a group of age-matched patients with no personal history of cancer. We found that our 70- to 92-year-old old cancer patients had significantly lower TREC levels in peripheral blood cells, a lower percentage of naïve and RTE $CD8^+$ T lymphocytes, and a more expanded $CD8^+$ memory cell subset than controls. Our results are partially consistent with those of a small trial in which head and neck cancer patients (most of them under 70 years old) revealed lower TREC levels than controls (Kuss, 2005). The expansion of memory $CD8^+$ cells is also consistent with the findings of another two recent studies conducted in breast cancer patients (Hueman, 2007; Poschke, 2012). The shift from naïve T cells to memory cells was probably due to a greater stimulation driven by tumor antigens. Since $CD8^+$ T cells are the key components of tumor immune surveillance, the tumor-induced dysfunction may be more evident in the CD8 cell subset than in the CD4 cell compartment (Klebanoff, 2006; Williams, 2007).

Our findings confirm the observations of a few other authors that thymic activity does not stop completely beyond 70 years of age (Ye, 2002, Nasi, 2006; Mitchell, 2010).

Notably, while TREC levels in cancer patients dropped significantly with increasing age, in controls they remained relatively constant. It may be that thymic output compensates for the loss of peripheral blood lymphocytes in elderly patients, but this homeostatic phenomenon seems to disappear in cancer patients. The shortage of larger studies on TRECs in people over 70 makes it difficult to say more on this issue.

In our study sample, the control group included a higher proportion of frail patients (according to the CGA), and had higher average MPI scores, and more chronic diseases and comorbidities than the cancer patients. Systemic inflammation and loss of peripheral blood cells may stimulate thymic output, which has an important role in immunological homeostasis. In fact, TREC levels correlated significantly with CIRS-CI in the control group, but not in the cancer patients. The imbalance between the two groups in terms of their geriatric conditions reinforces the magnitude of the significant difference observed in the cancer patients' and controls' TREC levels.

The hypothesis that immune activation can explain the finding of immune senescence in controls is supported by the CRP and (even more relevant) IL-6 levels, that were both found significantly higher in controls than in cancer patients. The role of IL-6, a circulating proinflammatory cytokine, as a marker of low-grade inflammation has been confirmed in various studies (Ferrucci, 1999; Ershler, 2000). Consistently with our data, its overproduction has been associated with a number of age-related illnesses and functional disabilities (Heikkila, 2008). In a few small studies, IL-6 levels were higher in cancer patients than in healthy controls (Vasto, 2006), whereas our opposite findings confirm the conclusions reached by other authors (Ershler, 2000; Heikkila, 2008) that large prospective studies are needed to clarify the role of IL-6, especially in elderly people.

While the hypothesis of immune homeostasis might justify a higher thymic output in controls, the age-related lower TREC levels seen in cancer patients may point to a pre-existing condition favoring immune escape and the onset of malignant disease. Be that as it may, the present exploratory study showed that cancer patients suffer from a more serious immune senescence than controls, but is unable to establish whether this is a consequence of cancer or related to a pre-existing immune impairment substrate facilitating the onset of cancer.

As concerns telomere length, a broad array of publications have explored the meaning of telomeres and telomerase activity in cancer patients, and there is plenty of data available on the relationship

between telomere length and aging-associated changes and chronic diseases (Wong, 2003; Aviv, 2006; Baird, 2006). On the other hand, telomere and telomerase behavior in the peripheral blood cells of elderly cancer patients was a matter that had yet to be explored. Our study showed that cancer patients' telomeres in PBMC are significantly shorter than those seen in controls. In addition, while control patients' telomeres became shorter with age, as expected on the strength of previous studies (Blasco, 2005; Steenstrup, 2013; Der, 2013), no such relationship between age and telomere length was seen in our cancer patients. This difference cannot be explained by a lower telomerase activity in cancer patients because telomerase activity was detected in the peripheral blood cells of most cancer patients, but virtually absent in those of most controls. The fact that our controls scored worse for the severity and comorbidity indexes than our cancer patients strongly emphasizes the magnitude of the difference in the two groups' telomere length.

While several studies have found shorter telomeres in tumor cells than in surrounding non-cancer cells (Bisoffi, 2006; Rampazzo, 2010), the intriguing finding of shorter telomeres in peripheral blood cells cannot be justified by the presence of cancer cells in the peripheral blood. Our cancer patients had radically-resected, early breast or colorectal cancer and had undergone a thorough radiological assessment to exclude either persistent local disease or distant metastases. As reported elsewhere (Franken, 2012; Shimada, 2012), the number of epithelial cells detectable in the peripheral blood in patients with early breast or colorectal cancer is so small that it cannot influence the telomere length or telomerase activity. We surmised that our control patients' longer telomeres related to this group's higher proportion of naïve-$CD8^+$ cells. To explore this issue, we separately estimated telomere length in CD8 naïve and CD8 memory cells from 3 cancer patients: the two subsets had a similar telomere length, suggesting that the shorter telomeres cannot be explained by an excessive replication/expansion of the memory cell subset (not shown). We therefore suggest that telomere length in peripheral blood reflects the more advanced biological aging of the immune system in cancer patients than in controls, a pre-existing condition that facilitated the onset of

cancer in the former. This hypothesis is in agreement with the findings of a case-control study involving 215 female lung cancer cases and 215 female controls. Authors reported a significant dose-response relationship between telomere length and risk of lung cancer (Lan, 2013). Also the results of a large trial involving more than 100.000 breast and ovarian cancer patients are consistent with the hypothesis that shorter telomeres predispose to increased cancer risk (Bojesen, 2013).

Neither thymic output markers nor telomere length correlated with the results of the CGA or MPI scores in our sample. There are no data in the literature on elderly cancer patients with which to compare our findings, while two other studies have explored the association between telomere length and frailty in elderly non-cancer patients: neither study found frailty indexes associated with telomere length (Collerton, 2012; Woo, 2008); and findings in our control group are consistent with their results. So, although telomere length is a marker of biological aging, their relationship cannot be extrapolated to the functional level represented by the geriatric assessment of frailty, largely contributed by several factors.

In conclusion, this exploratory study is the first to find that elderly cancer patients had significantly shorter telomeres in their peripheral blood cells than age-matched non-cancer patients. This result suggests the unpublished hypothesis – which would need to be tested in a larger study - that elderly people with shorter telomeres are at higher risk of developing cancer. Our elderly cancer patients also seemed to suffer from a more severe decline in thymic output and had a lower proportion of naïve $CD8^+$ cells than age-matched controls. It is noteworthy that the cancer patients' thymic output decreased significantly with increasing age, while telomere length in the peripheral blood cells was unassociated with age. Finally, this preliminary study also confirms that there is no relationship between the geriatric phenotypes investigated by the CGA and these molecular markers of aging.

Table 1A. Demographic and clinical characteristics of cancer patients and controls

	All patients, No. (%)	**Cancer patients,** No. (%)	**Controls,** No. (%)
Age (yrs)			
No.	91 (100)	52 (57.2)	39 (42.8)
Median, range	81, 70-92	81, 72-92	80, 70-91
Gender			
Male	26 (28.6)	19 (36.5)	7 (17.9)
Female	65 (71.4)	33 (63.5)	32 (82.1)
Performance status (ECOG)			
0-1	83 (91.2)	51 (98.1)	32 (82.1)
≥2	8 (8.8)	1 (1.9)	7 (17.9)
Social Condition			
Home	90 (98.9)	51 (98.1)	39 (100)
Nursing home	1 (1.1)	1 (1.9)	0 (0.0)
Type of Assistance			
Alone	23 (25.3)	9 (17.3)	14 (35.9)
Family	59 (64.8)	38 (73.1)	21 (53.8)
Others	9 (9.9)	5 (9.6)	4 (10.3)
Caregiver Assistance (hours/day)			
Median time, range	24, 2-24	24, 2-24	24, 3-24
Tumor Stage (TNM)			
I	-	16 (30.8)	-
II	-	33 (63.5)	-
III	-	3 (5.7)	-
Modality of Diagnosis			
Symptoms/Self examination	-	40 (76.9)	-
Screening	-	9 (17.3)	-
Incidental diagnosis	-	3 (5.8)	-
Therapeutical Choice			
Adjuvant therapy as for younger adults	-	31 (59.6)	-
Adapted tratment	-	18 (34.6)	-
No adjuvant therapy	-	3 (5.8)	-
Agreement to Proposed Therapy			
Ready	-	51 (98.1)	-
Patient refusal	-	1 (1.9)	-
Cause of Admission to hospital/ Outpatient Services			
Cardiovascular disease	-	-	18 (46.2)
Peripheral deep venous thrombosis	-	-	4 (10.2)
Gastrointestinal inflammatory disorders or bleeding	-	-	5 (12.8)
Screening of osteoporosis with no active comorbidity	-	-	12 (30.8)

Table 1B. Scores of geriatric tools in cancer patients and controls

Geriatric Tool	All patients	Cancer patients	Controls	OR (95% CI)	*p*-value
CGA, No. (%)					
Fit	37 (40.7)	24 (46.2)	13 (33.3)	1	
Vulnerable	28 (30.7)	19 (36.5)	9 (23.1)	1.14 (0.40-3.24)	0.801 [a]
Frail	26 (28.6)	9 (17.3)	17 (43.6)	0.29 (0.10-0.82)	0.020 [a]
MPI, No (%)					
Low	78 (85.7)	49 (94.2)	29 (74.3)	1	
Moderate	12 (13.2)	3 (5.8)	9 (23.1)	0.20 (0.05-0.79)	0.022 [a]
Severe	1 (1.1)	0 (0.0)	1 (2.6)	<0.01	ns [a]
MPI					
Median, range	0.25, 0.00-0.69	0.19, 0.00-0.44	0.25, 0.06-0.69	-	0.001 [a]
Dependencies in ADL, No (%)					
No	71 (78.0)	44 (84.6)	27 (69.2)	1	
≥1	20 (22.0)	8 (15.4)	12 (30.8)	0.41 (0.15-1.14)	0.790 [a]
Dependencies in IADL, No (%)					
No	57 (62.6)	36 (69.2)	21 (53.8)	1	
≥1	34 (37.4)	16 (30.8)	18 (46.2)	0.52 (0.22-1.23)	0.133 [a]
MMSE					
Median [IQR]	27.1 [24.4-28.4]	26.2 [24.4-27.3]	28.3 [26.4-29.3]	-	0.001 [b]
SPMSQ					
Median [IQR]	1.0 [0.0-2.0]	1.0 [0.0-2.0]	0.0 [0.0-2.0]	-	0.013 [b]
CIRS-CI					
Median [IQR]	3.0 [1.0-4.0]	2.0 [1.0-3.0]	3.0 [2.0-4.0]	-	0.032 [b]
CIRS-SI					
Median [IQR]	1.5 [1.3-1.6]	1.4 [1.2-1.5]	1.6 [1.4-1.8]	-	<0.001 [b]
GDS					
Median [IQR]	3.0 [1.0-6.0]	3.0 [1.2-6.0]	3.0 [1.0-6.0]	-	0.888 [b]
BMI					
Median [IQR]	25.7 [24.0-27.4]	25.8 [24.2-26.9]	25.0 [23.0-28.6]	-	0.911 [b]
Drugs for concomitant diseases					
Median [IQR]	5 [3-6]	4 [2-5]	5 [4-8]	-	0.003 [b]

[a] Pearson's χ^2 or Fisher test
[b] Mann-Whitney *U* test

Table 1C. Standard blood parameters: comparison between cancer patients and controls

Parameter	All patients Median [IQR]	Cancer patients Median [IQR]	Controls Median [IQR]	*p*-value [a]
Leucocytes (x10^9/l)	6.6 [5.3-7.9]	6.7 [5.5-7.9]	5.9 [5.0-7.5]	0.110
Lymphocytes (x10^9/l)	1.6 [1.3-1.9]	1.7 [1.3-1.9]	1.5 [1.2-1.9]	0.165
Neutrophils (x10^9/l)	3.8 [3.0-4.9]	3.9 [3.2-4.9]	3.6 [2.7-4.9]	0.141
Hemoglobin (g/l)	127.0 [114.0-140.0]	128.0 [111.0-142.0]	126.0 [116.0-138.0]	0.665
Platelets (x10^9/l)	236.0 [204.0-291.0]	253.5 [212.5-317.0]	225.0 [183.0-260.0]	0.011
Total plasmatic proteins (g/l)	66.8 [63.5-71.1]	68.4 [66.1-74.3]	64.0 [60.7-68.7]	<0.001
Albumin (%)	57.0 [54.0-59.6]	57.9 [54.7-60.6]	55.9 [53.9-58.2]	0.530
Gamma globulins (%)	15.2 [13.2-17.3]	14.9 [13.3-17.1]	15.8 [13.0-18.0]	0.549
Triglycerides (mmol/l)	1.1 [0.8-1.4]	1.2 [0.8-1.7]	0.9 [0.7-1.2]	0.009
Total cholesterol (mmol/l)	4.9 [3.9-5.8]	5.1 [4.3-5.7]	4.5 [3.7-5.8]	0.229
LDL-cholesterol (mmol/l)	2.8 [2.0-3.7]	3.3 [2.7-3.8]	2.4 [1.7-3.6]	0.006
HDL-cholesterol (mmol/l)	1.4 [1.1-1.9]	1.4 [1.1-1.7]	1.6 [1.1-1.9]	0.194
Creatinine (umol/l)	76.0 [68.0-93.0]	77.5 [68.2-95.0]	75.0 [68.0-93.0]	0.642
IgG (g/l)	10.5 [8.5-12.2]	10.3 [8.9-11.6]	11.0 [7.7-12.6]	0.934
IgA (g/l)	2.3 [1.8-2.9]	2.6 [1.8-3.4]	2.2 [1.6-2.7]	0.104
IgM (g/l)	0.9 [0.5-1.3]	0.8 [0.5-1.3]	0. 9 [0.5-1.4]	0.853
IL-6 (ng/l)	3.0 [1.9-5.3]	2.6 [1.9-4.3]	3.7 [2.1-6.3]	0.033
CRP (mmol/l)	3.5 [2.9-6.6]	3.5 [2.9-5.5]	3.5 [3.4-10.8]	0.044

CMV [b]				
***n* positive/*n* tested (%)**	88/91 (96.7%)	49/52 (94.2%)	39/39 (100%)	0.498
IgG (UI/ml)	15000 [8200-21000]	15000 [8125-19750]	15000 [9300-24000]	

[a] Mann-Whitney *U* test
[b] CMV serology: Serum CMV antibody (IgG) titers (Enzygnost® Anti-CMV/IgG) >230 were considered to be positive.

Table 1D. Thymic output and telomere length in cancer patients and controls

Parameter	All patients Median [IQR]	Cancer patients Median [IQR]	Controls Median [IQR]	*p*-value
% $CD3^+$	54.3 [40.8-65.2]	53.6 [37.8-65.5]	56.3 [41.8-65.2]	0.560 [a]
% $CD4^+$	35.5 [25.6-49.1]	35.0 [21.1-46.7]	35.6 [26.6-51.8]	0.389 [a]
% $CD8^+$	15.7 [10.1-22.4]	15.4 [9.7-22.6]	16.4 [10.5-22.8]	0.717 [a]
% naïve, $CD4^+CD45RA^+$	37.4 [27.2-51.3]	36.0 [24.2-50.6]	40.0 [30.2-53.0]	0.137 [a]
% naïve, $CD8^+CD45RA^+$	54.1 [45.0-65.2]	50.2 [39.8-62.9]	55.7 [52.6-65.8]	0.016 [a]
% RTE, $CD4^+CD45RA^+CD31^+$	21.3 [14.2-33.4]	20.3 [10.4-32.0]	23.3 [16.8-33.5]	0.176 [a]
% RTE, $CD8^+CD45RA^+CD31^+$	37.6 [29.1-48.9]	34.2 [24.7-46.4]	44.9 [36.0-50.7]	0.004 [a]
TREC copy number/10^5 PBMC	19.0 [10.1-37.0]	16.0 [7.7-31.5]	25.0 [14.0-56.0]	0.031 [a]
Telomere length (T/S ratio)[c]	1.15 ± 0.30	1.09 ± 0.31	1.22 ± 0.30	0.046 [b]
Telomerase activity				
No. positive/ No. tested	58/90	38/52	20/38	
Relative Units	5.6 [0.0-26.0]	14.6 [0.0-36.8]	1.4 [0.0-6.4]	0.003 [a]

[a] Mann-Whitney *U* test
[b] Student *t*-test
[c] Data are expressed as mean ± Standard Deviation

Table 1E. TREC and telomere profile on cancer patients and controls

	Cancer Patients No. (%)	**Controls** No. (%)	**OR (95% CI)**	***p*-value**
TREC levels [a]				
TREC high	20 (40.0%)	23 (62.2%)	1	
TREC low	30 (60.0%)	14 (37.8%)	2.46 (1.03-5.90)	0.043
Telomere length [b]				
T/S long	21 (40.4%)	23 (62.2%)	1	
T/S short	31 (59.6%)	14 (37.8%)	2.43 (1.02-5.76)	0.045
TREC levels & Telomere length				
Others [c]	31 (62.0%)	30 (85.7%)	1	
TREC low & T/S short	19 (38.0%)	5 (14.3%)	3.68 (1.22-11.11)	0.021

[a] TREC low: TREC ≤ median, TREC high: TREC > median
[b] T/S short: T/S ≤ median, T/S long: T/S > median (the median value is coincident with the mean value)
[c] Others: TREC high & T/S long, TREC low & T/S long and TREC high & T/S short

Table 1F. Relationship between markers of immune senescence and geriatric scores in cancer patients and controls

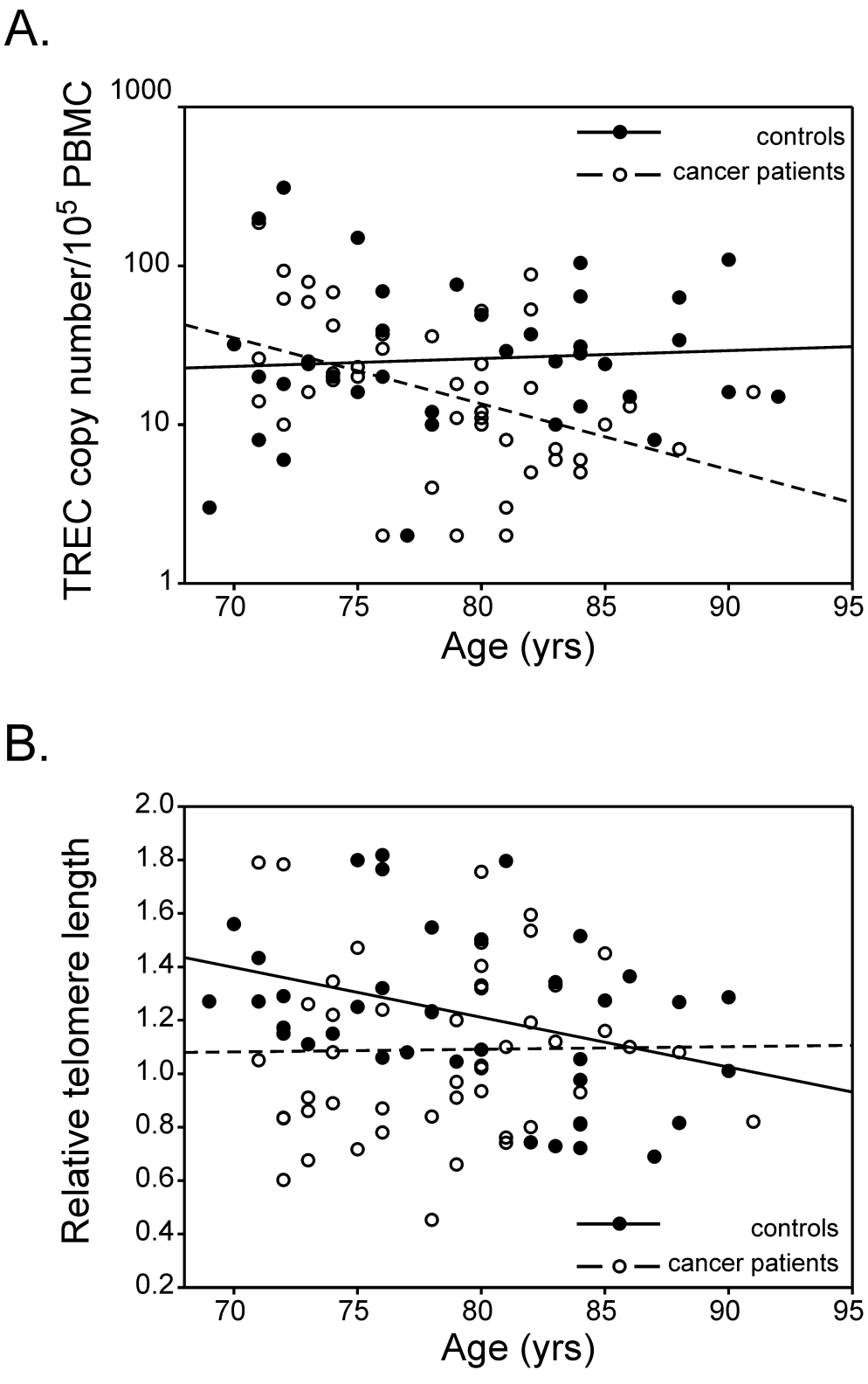
	TREC level			Telomere length			Telomerase activity		
	All patients	**Cancer patients**	**Controls**	**All patients**	**Cancer patients**	**Controls**	**All patients**	**Cancer patients**	**Controls**
MPI	-0.03, 0.77	-0.04, 0.78	-0.24, 0.16	-0.04, 0.74	0.01, 0.95	-0.26, 0.13	-0.03, 0.81	0.16, 0.25	0.00, 0.98
MMSE	-0.1, 0.35	-0.28, 0.05*	-0.14, 0.42	0.06, 0.61	0.15, 0.28	-0.31, 0.06	-0.14, 0.19	-0.17, 0.22	0.19, 0.25
CIRS-CI	-0.04, 0.71	0.04, 0.79	-0.31, 0.07	0.04, 0.70	0.05, 0.72	-0.05, 0.76	-0.12, 0.26	0.01, 0.94	-0.18, 0.27
CIRS-SI	0.26, 0.02*	-0.07, 0.61	0.45, 0.01**	0.16, 0.13	-0.11, 0.42	0.35, 0.03*	-0.06, 0.60	0.01, 0.95	0.18, 0.27
GDS	-0.03, 0.79	-0.19, 0.19	0.22, 0.18	-0.10, 0.34	-0.09, 0.54	-0.05, 0.75	-0.24, 0.02*	-0.20, 0.15	-0.26, 0.12
BMI	-0.03, 0.79	0.01, 0.96	-0.10, 0.57	0.02, 0.83	0.14, 0.31	-0.11, 0.53	0.27, 0.01**	0.29, 0.04*	0.14, 0.39
Concomitant drugs	-0.01, 0.91	0.11, 0.43	-0.39, 0.02*	-0.13, 0.24	-0.18, 0.21	-0.24,0.15	-0.02, 0.89	0.13, 0.36	0.08, 0.65

Data are expressed as Spearman's rho coefficient, *p*-value.

* Correlation is significant at the level 0.05 (two-tailed)

** Correlation is significant at the level 0.01 (two-tailed)

Fig. 1A. Correlation between age and (A) TREC levels and (B) telomere lengths in cancer patients and controls. Panel (A): TREC levels decline with age in peripheral blood cells of cancer patients (r = −0.478, p b 0.001), but not in controls (r = 0.071, p = 0.677). Panel (B): telomere lengths decrease with age in peripheral blood cells of controls (r = −0.354, p = 0.031), but not in cancer patients (r = −0.011, p = 0.938).



CHAPTER 2

The Mortality Risk

DOES THE MULTIDIMENSIONAL PROGNOSTIC INDEX (MPI), BASED ON A COMPREHENSIVE GERIATRIC ASSESSMENT (CGA), PREDICT MORTALITY IN CANCER PATIENTS? RESULTS OF A PROSPECTIVE OBSERVATIONAL TRIAL

2.1 BACKGROUND AND AIMS

A large proportion of cancer patients are at least 70 yrs old (Siegel, 2012). In recent decades, strong evidence has emerged to indicate that age alone is not an absolute contraindication for cancer treatments. Instead of chronological age, a patient's biological age - estimated by means of a thorough geriatric assessment - should drive any therapeutic decisions (Balducci, 2003).

In fact, the complexity of geriatric and oncological parameters and their interactions are such that proposing anticancer treatment for these elderly patients means entering a controversial field. On the one hand, oncologists must deal with the risk of cancer-related morbidity and mortality, while on the other they have to consider the patient's life expectancy irrespective of their neoplasm (Extermann, 2005; Surbone, 2007).

Given the serious difficulty of managing older cancer patients, for some years now numerous oncologists (Extermann, 2004; Basso, 2007; Marenco, 2008;) have been focusing on an approach to cancer patients based on a Comprehensive Geriatric Assessment (CGA), because the importance of evaluating the geriatric aspects as well as those strictly related to the neoplastic disease is becoming increasingly clear.

The CGA was first introduced in geriatric clinics to identify potential causes of frailty because frail patients are often heterogeneous in their clinical presentation and complicated to care for (Extermann, 2003; balducci, 2000). The CGA is now widely used in everyday clinical geriatric practice to deal with patients who have concomitant diseases and different functional conditions (Ferrucci, 2001). This tool is used not only for the baseline assessment and diagnosis, but also for serial, prospective patient management. Since one of its main features is that it is interdisciplinary, the CGA is a useful tool for investigating the frail (Rubenstein, 2004; frees, 2012).

In the context of geriatric oncology, the CGA has proved essential for distinguishing patients who are fit and deserve the same active oncological treatment as younger adults from vulnerable patients

eligible for an adapted treatment with lower doses of drugs and strict monitoring, and frail patients too sick to receive treatments other than the best supportive care (Balducci, 2007).

Since the pioneering times of geriatric oncology in the 1980s, the concept of CGA has progressed and the Multidimensional Prognostic Index (MPI), based on a geriatric assessment (Pilotto, 2005; Pilotto, 2008), has been developed. The MPI assesses 8 domains derived from geriatric scales widely used in clinical practice, relating to functional, cognitive and nutritional conditions, comorbidities and pressure sore risk. It also considers clinical issues such as medication, and social aspects too. The MPI has been validated in two independent cohorts of elderly hospitalized patients (Pilotto, 2005): a worse MPI was positively associated with a higher risk of mortality. The prognostic value of the MPI has been confirmed in cohorts of hospitalized patients with acute or re-emerging chronic diseases, such as pneumonia, dementia, gastrointestinal bleeding, heart failure, and metabolic syndrome (Pilotto, 1995; Pilotto, 2007; Pilotto, 2009).

Yourman et al (Yourman, 2012) recently conducted a systematic assessment of the qualities and limitations of various disease-specific prognostic indices for all-cause mortality in older adults. Among the indices validated for hospitalized patients, the MPI was judged to be well calibrated and demonstrated a good discrimination in the validation cohort. The MPI has never been validated for the purposes of predicting mortality in cancer patients, however, where the need for a prognostic index that does not only consider the patient's oncological features is strongly felt (Monfardini, 2012).

The main aim of the present study was to ascertain the prognostic value of the MPI at 6 and 12 months in geriatric cancer patients. A second aim was to investigate whether other geriatric issues not considered in the MPI, but extremely relevant in the traditional CGA, could strengthen the prognostic value of the MPI when applied to cancer patients.

2.2 PATIENTS AND METHODS

2.2.1 Study population

Patients aged 70 yrs and older admitted between 17 April 2008 and 19 April 2011 to the Geriatric Clinic, Geriatric Surgery Clinic and Medical Oncology Unit at Padua Hospital with a recently-diagnosed cancer were screened for eligibility.

Our inclusion criteria were as follows: 1) histologically confirmed diagnosis of inoperable, locally advanced or metastatic solid cancer; 2) ability to provide informed consent or availability of a legally-allowable representative; 3) feasibility of obtaining a CGA at enrollment; 4) willingness of patients and their caregivers to answer questionnaires. Patients presenting with a cognitive impairment severe enough to hamper communication with physicians were excluded by the study.

The following details were collected at the baseline: date of birth, gender, formal education, weight, height, body mass index (BMI), body surface area (BSA), calculated as ($W^{0.425} \times H^{0.725}$) x 0.007184) (26), clinical history, and past and present medication, current pathologies, sites of primary cancer, any metastases, and cancer treatments received.

All patients submitted to a standard CGA at the time of their first oncological visit as reported below. Living status was assessed by directly contacting the participants (or their caregivers), or consulting the *Registry Offices* of the cities where the patients resided; dates of death were obtained from death certificates.

The present study was approved by our Institutional Ethics Committee and conducted according to the Declaration of Helsinki and the guidelines of Good Clinical Practice. Written informed consent was obtained from patients or the relatives of the demented or critically ill prior to their enrollment in the study.

2.2.2 The Comprehensive Geriatric Assessment (CGA)

The CGA was administered by a multidisciplinary team that included a medical oncologist, geriatricians and psychologists, at the time of first admission to the referring units due to a diagnosis of cancer.

Functional status was evaluated using the Activities of Daily Living (ADL; Katz, 1970) and Instrumental Activities of Daily Living (IADL; Lawton, 1969) scales. ***Cognitive Status*** was assessed with the Short Portable Mental Status Questionnaire (SPMSQ; Pfeiffer, 1975) and Mini Mental Status Examination (MMSE; Pfeiffer, 1975; Folstein, 1975).

Comorbidities and their severity were examined using the Cumulative Illness Rating Scale-Comorbidity Index (CIRS-CI) and the Cumulative Illness Rating Scale-Severity Index (CIRS-SI) (Conwell, 1993). ***Nutritional Status*** was explored with the Mini Nutritional Assessment (MNA) (Guigoz, 1999). ***The risk of developing pressure sores*** was tested with the Exton Smith Scale (ESS) (Blitz, 1966). ***Medication*** taken was defined as the number of different drugs used by patients for concomitant diseases and disorders at the time of admission. ***Social aspects*** included household composition and home services, and institutionalization was also noted.

In addition to the MPI, other geriatric aspects were considered, including: ***affective status,*** evaluated with the Geriatric Depression Scale (GDS; Yesavage, 1983); ***self-reported pain,*** assessed with the Visual Numeric Scale (VNS; Ritter, 2006); and the ***burden of care for caregivers***, measured with the Caregiver Burden Inventory (CBI; Novak, 1989).

2.2.3 The Multidimensional Prognostic Index (MPI)

The MPI score was calculated for each patient from the results of the various tests (ADL, IADL, SPMSQ, CIRS-CI, MNA, ESS, number of drugs, and social conditions), as reported elsewhere by Pilotto et al (Pilotto, 2007; Pilotto, 2009; Pilotto, 2010). As in their original publications, three grades of severity risk were also considered to better stratify the study population (Table 2A), i.e.

low mortality risk (MPI ≤ 0.33), moderate risk (MPI between 0.34 and 0.66), and high risk (MPI > 0.66).

2.2.4 Statistical analysis

The analyses were performed using SAS (9.2) statistical software from the SAS Institute, Cary (NC).

The distribution of the demographic and clinical variables between the men and women, and across the MPI scores was summarized in the form of means and standard deviations (SD) or frequencies. Comparisons between groups and categorical variables were assessed with the chi-square or Fisher's exact test (two-tailed). Differences between the means for groups were analyzed, if normally distributed, with the Generalized Linear Models (GLM) procedure, after testing for homoscedasticity with Levene's test (and Welch's test was performed when this assumption was not confirmed); if the differences were not normally distributed, non-parametric (Mann-Whitney U, Kruskal-Wallis) tests were used.

The associations between the time to death at the 6- or 12-months follow-up and the MPI scores, individual MPI domains, and clinical characteristics was analyzed using a Cox's proportional hazards regression model adjusted for age and gender. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated to estimate the strength of the associations. Multivariate Cox's regression analyses with a stepwise procedure (considering a threshold level of 0.15) were used to identify other possible mortality predictors (formal education, BSA, CIRS-SI, MMSE, GDS, VNS, CBI, site of primary tumor, metastases, cancer treatments), in addition to the MPI. Observation time was calculated as the time elapsing from admission to death or the end of the follow-up, whichever came first. The assumption of proportionality was assessed by analyzing the Schoenfeld residuals of the covariates introduced in the model. Age- and sex-adjusted survival curves were also derived from the Cox's regression.

The discriminatory power of the mortality model at 6 and 12 months of follow-up was assessed by calculating the area under the ROC curves for the MPI (considered as a continuous variable) using logistic regression models.

A p value <0.05 was considered statistically significant.

2.3 RESULTS

2.3.1 Characteristics of study population

Of the 171 patients screened using our inclusion criteria, 11 patients were excluded because: they refused to take part in the study (3 patients); they were unable to complete the CGA (6 patients); or they could not sign the informed consent and had no legally-allowable representative (2 patients). The final study population thus included 160 patients, 88 females (55%) and 72 males (45%), with a mean age of 79.4 ± 5.7 years (range 69-93), and a mean formal education 6.4 ± 3.6 years (range 0-19).

Histological confirmation of the diagnosis of cancer was not available for 4 patients (2.5%) whose low performance status contraindicated any invasive diagnostic procedures. The diagnosis of cancer was judged highly likely in these patients, however, based on specific physical or radiological findings.

The baseline characteristics of the study population by gender are shown in Table 2.B. There were significant gender-related differences in the sample's weight, height, BSA, GDS, CIRS-SI, CIRS-CI, site of primary tumor, endocrine therapy, and social conditions. The overall mortality rate was 34.4% (55 patients) at 6 months and 46.9% (75 patients) at 12 months of follow-up, with no significant differences between males and females.

2.3.2 Distribution by MPI

The main features of the study population by MPI score are shown in Table 2.C. Ninety-six patients (60%) had a low MPI, 48 (30%) a moderate MPI and 16 (10%) a severe MPI. The MPI scores were significantly associated with all the single MPI domains, age, weight, BMI, BSA, CIRS-SI, MMSE, GDS, VNS, CBI, and site of primary tumor. No significant associations emerged between MPI and formal education, gender, height, metastases and any oncological treatments.

2.3.3 Prognostic value of the MPI

Increasing mortality rates after 6 and 12 months of follow-up coincided with higher MPI scores (see Table 2.C).

In the univariate Cox's regression analyses, MPI adjusted for age and sex, most of its individual components (ADL, IADL, MNA, CIRS-CI, ESS), and also BSA, CIRS-SI, GDS, VNS, CBI, site of primary tumor, and presence of metastases, were significant predictors of mortality at 6 and 12 months of follow-up (Table 2.D).

The MPI-related hazard ratios were higher at 6 months of follow-up than at 12 months, a high MPI being associated with a HR of 8.094 (95% CI 3.749-17.475, $p<0.0001$) at 6 months as opposed to 5.655 (95% CI 2.866-11.158, $p<0.0001$) at 12 months. When the MPI was considered as a continuous variable, any increase by 0.2 units (corresponding to the lower quartile) was associated with a 2.347-fold increase in the mortality risk (95% CI=1.838-2.997) at 6 months and a 2.051-fold increase (95% CI=1.662-2.531) at 12 months. Figure 2A shows the age- and sex-adjusted survival curves for the three subgroups with low, moderate and high MPI scores; patients with high MPI scores had a significantly higher mortality rate than patients with lower MPI scores ($p<0.0001$).

Age- and sex-adjusted multivariate Cox's regression analyses conducted to assess the potential mortality risk factors (with MPI as a continuous variable, formal education, BSA, CIRS-SI, MMSE, GDS, VNS, CBI, site of primary tumor, metastases, and oncological treatments) at the two time

points are presented in Table 2.E. MPI, CIRS-SI, BSA, GDS, MMSE, chemotherapy and a diagnosis of primary lung cancer were associated with mortality at 6 and 12 months.

The discriminatory power of the MPI's predictive performance was statistically significant (Table 2.F).

The age- and sex-adjusted area under the ROC curve for MPI score at 6 and 12 months of follow-up were 0.81 (95% CI, 0.74-0.88) and 0.78 (95% CI, 0.71-0.85), respectively. The prognostic performance of the MPI was also assessed in a regression model adjusted for age, sex and the other mortality predictors verified at the multivariate analysis. This last model seems better able to predict mortality both at 6 months (area under the ROC curve =0.94, 95% CI 0,869-0.959, $p<0.0001$) and 12 months (area under the ROC curve =0.87, 95% CI 0,819-0.928, $p<0.0001$) of follow-up (Table 2.F).

2.4 DISCUSSION

To our knowledge, this is the first prospective study to evaluate the prognostic value of the MPI in a cohort of ECP. According to the current practice guidelines of the major international societies of oncology, some form of geriatric assessment applied to ECP should be mandatory before any major treatment decisions are made (Surbone, 2007, Extermann, 2005) in order to estimate their life expectancy irrespective of their tumor and to explore their functional reserves and the risk of treatment complications. There is still no consensus on the best form of geriatric assessment in the oncological setting (Falci, 2011). One of the main drawbacks of applying a conventional CGA to oncological patients lies in its lack of standardization. A number of investigators have developed scoring systems, based in various ways on the CGA, to estimate short- and long-term mortality. Among the short-term mortality predictors, the MPI is probably the most appealing because it summarizes the information obtainable from most of the items in the CGA in a single score ranging from 0 to 1 (Pilotto, 2008).

This study showed that the MPI significantly predicted all-cause mortality at 6 and 12 months in a cohort of elderly patients with locally advanced or metastatic disease. At the multivariate Cox's regression analysis, the MPI showed the highest hazard ratio, even compared with the patients' oncological features.

This finding is extremely important, in our opinion, because it confirms that the prognosis is not influenced by the oncological picture alone in elderly adults with advanced cancer, but also by the their geriatric features (most of which are included in the MPI).

Given that solid cancers in an advanced stage are well known to carry an unfavorable prognosis, some might argue that this selection criterion dramatically impairs the utility of testing a prognostic index such as the MPI. Conversely, our results indicate that the presence of advanced cancer does not *per se* impair the prognostic value of the MPI.

This finding is consistent with results reported by Basso et al, who recently evaluated the prognostic meaning of traditional CGA in more than 800 cancer patients: their status according to the CGA predicted a different survival for different groups in the cohort of patients as a whole, and significance was maintained for the differences between the subgroups of patients in the adjuvant/neoadjuvant setting and those with advanced disease (Basso, 2011).

A number of conventional geriatric characteristics also retained their prognostic value in our cohort of elderly patients with advanced or metastatic cancer.

In fact, in our study population as in Pilotto's cohorts of, living alone was significantly associated with a worse MPI score: this relationship is explained by the fact that solitude is often associated with depression, a higher risk of falls, malnutrition, and financial problems; all these factors could influence a patient's functional and clinical conditions. Solitude has proved to be a negative prognostic factor in a number of geriatric and oncological trials (Reuben, 1992; Kroenke, 2006). In the oncological setting, there is the added justification relating to the higher risk of treatment complications occurring in the absence of an active caregiver. This is an important issue in the

management of ECP and a potential target of intervention: preventing isolation could in itself improve the outcome in ECP.

The burden of comorbidities, defined as the total number of associated diseases and the CIRS severity index, was significantly associated with the MPI score. Previous studies had already suggested the important prognostic value of comorbidities (Chen, 2007; Cudennec, 2009) in elderly patients, especially in association with functional decline (Zagonel, 2002). While comorbidity, as expressed by the CIRS-CI, seemed to be a good prognostic tool in geriatric patients, in this cohort of ECP the severity of their concomitant diseases and disorders (expressed by the CIRS-SI) became important. The present study showed that associated diseases retain their prognostic value in elderly patients with cancer too. Since it is essential to consider a patient's comorbidities and their severity before preparing an oncological treatment plan – given the direct contraindications to the use of certain drugs and the impact on the patient's life expectancy (Hurria, 2011) - this finding enhances the value of the MPI and its applicability in daily clinical practice.

A significant association was likewise found between the MPI score and the ADL and IADL scores. For a long time now, functional status has been known to be an important risk factor in cancer patients (Wedding, 2007). In addition to its direct effect on overall survival, cancer patients with disabilities are less likely to receive a precise diagnosis and optimal treatment, and this has a further negative effect on their survival (Basso, 2011).

Significant relationships were also found between decreasing body weight, decreasing BMI and worsening nutritional status and a worsening MPI score. Malnutrition is an important problem in cancer patients, because cancer itself causes loss of appetite and is implicated in the development of cachexia, and this influences the feasibility of cancer treatments. In elderly patients, functional and cognitive decline also raise the risk of malnutrition, and this condition further reduces the chances of treating their cancer (De Groot, 1998).

Even when oncological treatments are possible, patients' poor general conditions could reduce their tolerance of such therapy. Oncologic treatments can influence weight by causing vomiting and

changing the taste of foods, as shown in previous studies on the prognostic value of the MNA (De Groot, 1998).

Limited mobilization is the main cause and consequence of functional decline that exposes patients to a higher risk of bed sores (Takahashi, 2008). By further impairing a patient's mobility and nutritional status, cancer and its treatments may also contribute to the risk of bed scores, although no studies have explored the prognostic value of the Exton Smith Score in cancer patients. Bed sores are predictors of a higher mortality risk even in istituzionalized patients. In the present study, the patients' ESS scores were consistent with their MPI scores and were of prognostic value, good ESS scores being associated with a 19% reduction in the 12-month mortality rate (HR 0.816, 95% CI 0.762-0.875, $p<0.0001$).

Our cohort's MPI scores correlated strongly with some of the geriatric tests not originally included in the prognostic index, i.e. the MMSE, GDS, CIRS-SI, VNS and CBI. The independent prognostic value of these geriatric tests was explored at both univariate and multivariate analysis.

GDS emerged as a significant prognostic factor, patients with GDS ≥ 5 carrying a 3.6-fold higher mortality risk at 6 months. This result confirms the importance of assessing and treating depression in ECP with a view to improving the patients' compliance with care and monitoring their perception of chemotherapy-related adverse events.

The CBI was also found to predict 6- and 12-month mortality at univariate analysis, but its prognostic value was not confirmed at multivariate analysis, and the same applied to pain levels measured with the VNS.

Surprisingly, the severity index calculated with the CIRS (not included in the MPI model) was associated with a relevantly higher mortality risk and retained its prognostic significance at multivariate analysis.

The assessment of predictors not included in the MPI model is not just an academic exercise, it can pave the way to an integration of the MPI for use in cancer patients. This possibility is supported by the AUC in the ROC curves that we calculated, in which adjusting the MPI model not only for age

and gender, but also for CIRS-SI, MMSE, BSA, GDS, chemotherapy and a diagnosis of lung cancer, coincided with the best MPI performance, with an appreciable area under the ROC curve of 0.914 at 6 months.

The present study has some limitations. The sample size was relatively small considering the heterogeneity of the study population. In defining our inclusion criteria, we chose to consider a study population comparable with those observed in daily clinical practice because one of the most important criticisms of trials on ECP concerns their marked selectivity, which makes it difficult to transfer the results to routine clinical activity (Scher, 2012). Of course, our exploratory study cannot answer the question of which geriatric assessment is best in the context of geriatric oncology, but it certainly demonstrates that the MPI can be extremely useful in daily clinical practice for estimating overall survival and supporting oncological treatment decisions relating to elderly adults with locally advanced or metastatic cancer.

Further studies on larger and more homogenous populations are needed to thoroughly assess the role of MPI in single cancer types, in the early stages of cancer disease, and in the setting of outpatient health care services. In the research setting, thanks to the marked standardization of the method and its clear clinimetric properties, the MPI may be applied more profitably than the traditional CGA in the context of clinical trials specifically designed for ECP (Balducci, 2003; Extermann, 2004).

In conclusion, the present study confirms the prognostic value of the MPI in elderly patients with advanced cancer. In this specific setting, the MPI retained the same reliability and accuracy as reported in the original study by Pilotto et al. This index may consequently be used in daily clinical practice for the proper risk assessment of elderly patients with advanced cancer who are potential candidates for active oncological treatments. Further studies are nonetheless needed to confirm these findings in larger populations and assess the potential for integrating the MPI with the CIRS severity index and the GDS.

Table 2A Multidimensional Prognostic Index (MPI) score assigned to each domain based on the severity of the problems

Assessment	**Problems**		
	No (value = 0)	**Minor (value = 0.5)**	**Severe (value = 1)**
Activities of Daily Living (ADL)[a]	6–5	4–3	2–0
Instrumental IADL (IADL)[a]	8–6	5–4	3–0
Short Portable Mental Status Questionnaire (SPMSQ)[b]	0–3	4–7	8–10
Comorbidity-Index (CIRS-CI)c	0	1–2	≥3
Mini Nutritional Assessment (MNA)[d]	≥24	17–23.5	<17
Exton–Smith Scale (ESS)[e]	16–20	10–15	5–9
Number of Drugs	0–3	4–6	≥7
Social Condition	Living with family	Institutionalized	Living alone

[a] Number of active functional activities,
[b] number of errors,
[c] number of diseases,
[d] MNA score: ≥24 = satisfactory nutritional status; 17–23.5 = at risk of malnutrition; <17 = malnutrition,
[e] ESS score: 16–20 = minimum risk; 10–15 = moderate risk; 5–9 = high risk of developing scores

Table 2B: Characteristics of subjects by gender

	Females (n = 88)	Males (n = 72)	*p*-value	All (n=160)
General and oncological characteristics				
Age	80.1 ± 5.7 (70-93)	78.4 ± 5.6 (69-92)	0.0729	79.4 ± 5.7 (69-93)
Educational level (years)	6.0 ± 3.2 (0-18)	6.9 ± 4.1 (0-19)	0.5547	6.4 ± 3.6 (0-19)
Weight (kg)	61.9 ± 12.3 (30- 95)	73.6 ± 12.4 (47-103)	<0.0001	67.2 ± 13.6 (30-103)
Height (cm)	158.2 ± 7.3 (135-182)	172.0 ± 6.3 (155-185)	<0.0001	164.5 ± 9.8 (135-185)
BMI (Kg/m^2)	24.7 ± 4.5 (15.5-42.2)	24.8 ± 3.7 (16.1-34.4)	0.8502	24.7 ± 4.2 (15.5-42.2)
BSA (m^2)	1.6 ± 0.2 (1.1-2.1)	1.9 ± 0.2 (1.5-2.2)	<0.0001	1.7 ± 0.2 (1.1-2.2)
CIRS-SI	1.7 ± 0.4 (1.1-3.1)	2.0 ± 0.5 (1.2-3.2)	0.0011	1.8 ± 0.4 (1.1-3.2)
MMSE	23.3 ± 5.6 (9-30)	24.3 ± 4.4 (7-30)	0.5349	23.8 ± 5.1 (7-30)
GDS	5.2 ± 3.1 (0-13)	4.3 ± 3.4 (0-12)	0.041	4.8 ± 3.3 (0-13)
VNS	2.1 ± 2.7 (0-10)	2.9 ± 3.1 (0-10)	0.1226	2.5 ± 2.9 (0-10)
CBI	7.5 ± 11.3 (0-47)	10.2 ± 13.5 (0-53)	0.1214	8.7 ± 12.4 (0-53)
Site of primitive tumor			<0.0001	
Lung	3.4	31.9		16.3
Stomach/Esophagus	13.6	8.3		11.3
Breast	37.5	1.4		21.3
Colon-rectal	31.8	40.3		35.6
Other sites	13.6	18.1		15.6
Metastasis	58.1	62.5	0.5772	60.1
Oncological Treatments				
Surgery	31.8	25.0	0.3431	28.8
Chemotherapy	46.6	52.8	0.4361	49.4
Endocrine-therapy	17.1	1.4	0.0009	10.0
Radiotherapy	8.0	1.4	0.2256	10.0
None	9.1	20.8	0.0352	14.4
MPI Domains				
MNA	20.2 ± 5.5 (6.5-29)	20.7 ± 5.7 (8-29)	0.4208	20.4 ± 5.6 (6.5-29)
ADL	4.7 ± 2.1 (0-6)	4.5 ± 2.0 (0-6)	0.3923	4.6 ± 2.0 (0-6)
IADL	5.3± 2.8 (0-8)	5.8 ± 2.6 (0-8)	0.2811	5.5 ± 2.7 (0-8)
CIRS-CI	2.9 ± 1.6 (0-8)	4.2 ± 2.1 (1-9)	0.0002	3.5 ± 1.9 (0-9)
ESS	17.1 ± 2.8 (9-20)	17.5 ± 2.8 (8-20)	0.4461	17.3 ± 2.8 (8-20)
SPMSQ	1.8 ± 2.1 (0-9)	1.5 ± 2.0 (0-10)	0.2377	1.7 ± 2.1 (0-10)
Social Condition			0.0010	
Living alone	35.2	12.5		25.0
Living with family	61.4	86.1		72.5
Institutionalized	3.4	1.4		2.5
Number of Drugs			0.2392	
0-3 drugs	52.3	38.9		46.3
4-6 drugs	33.0	41.7		36.9
≥ 7 drugs	14.8	19.4		16.9
MPI	0.35 ± 0.22 (0.06-0.88)	0.34 ± 0.20 (0.06-0.75)	0.7919	0.35 ± 0.21 (0.06-0.88)
Mortality				
6 months	33.0	36.1	0.6758	34.4
12 months	46.6	47.2	0.9365	46.9

Data are presented as % or mean±SD (min-max)
NS: not significant differences
Abbreviation: BMI, Body Max Index; BSA, Body Surface Area; CIRS-SI, Cumulative Illness Rating Scale-Severity Index; MMSE Mini-Mental State Examination, GDS Geriatric Depression Scale short form, VNS Visual Numeric Scale, CBI Caregiver Burden Inventory, MNA, Mini Nutritional Assessment; ADL, Activities of Daily Living; IADL, Instrumental Activities of Daily Living; CIRS-CI, Cumulative Illness Rating Scale-Comorbidity Index; ESS, Exton Smith Scale; SPMSQ, Short Portable Mental Status Questionnaire; MPI, Multidimensional Prognostic Index.

Table 2C: Characteristics of subjects according to MPI grade

	MPI			
	Low (n = 96)	**Moderate (n = 48)**	**Severe (n = 16)**	***p*-value**
General and oncological characteristics				
Age	78.2 ± 4.9 (69-89)	81.3 ± 6.0 (71-92)	81.0 ± 7.5 (69-93)	0.0089
Gender				0.2170
Female	57.3	45.8	68.8	
Male	42.7	54.2	31.2	
Educational Level (ys)	6.2 ± 3.3 (0-19)	6.1 ± 3.7 (0-19)	8.6 ± 4.9 (3-18)	0.1425
Weight (Kg)	70.2 ± 13.5 (40-102)	63.5 ± 13.3 (30-103)	60.2 ± 9.8 (34.8-75)	0.0018
Height (cm)	164.5 ± 9.8 (145-185)	164.4 ± 10.0 (135-182)	164.7 ± 9.6 (150-180)	0.9752
BMI (Kg/m^2)	25.8 ± 4.1 (17.6-42.2)	23.4 ± 3.9 (16.1-34.4)	22.1 ± 2.7 (15.5-27.3)	<0.0001
BSA (m^2)	1.8 ± 0.2 (1.3-2.2)	1.7 ± 0.2 (1.1-2.2)	1.7 ± 0.2 (1.2-1.9)	0.0362
CIRS-SI	1.7 ± 0.4 (1.1-3.2)	2.0 ± 0.5 (1.2-3.1)	2.1 ± 0.5 (1.3-3.1)	0.0002
MMSE	25.5 ± 4.0 (14-30)	22.9 ± 5.0 (7-30)	17.4 ± 5.5 (9-27)	<0.0001
GDS	3.8 ± 2.9 (0-12)	5.8 ± 3.2 (0-12)	7.5 ± 3.5 (0-13)	<0.0001
VNS	1.6 ± 2.3 (0-8)	3.4 ± 2.9 (0-10)	4.4 ± 4.1 (0-10)	0.0001
CBI	4.0 ± 7.3 (0-33)	13.5 ± 14.4 (0-53)	23.3 ± 14.3 (0-48)	<0.0001
Site of primitive tumor				<0.0001
Lung	13.5	22.9	12.5	
Stomach/Esophagus	9.4	12.5	18.8	
Breast	28.1	12.5	6.3	
Colon-rectal	40.6	27.1	31.3	
Other sites	8.3	25.0	31.3	
Metastasis	63.5	54.2	50.0	0.5464
Oncological Treatments				
Surgery	25.0	31.3	43.8	0.2776
Chemotherapy	55.2	41.7	37.5	0.1873
Endocrine-therapy	12.5	8.3	0.0	0.3514
Radiotherapy	7.3	18.8	6.3	0.0894
None	89.6	81.3	75.0	0.1651
MPI Domains				
MNA	23.2 ± 3.7 (13.0-29.0)	16.9 ± 5.3 (6.5-25.0)	13.8 ± 4.5 (8.5-23.0)	<0.0001
ADL	5.9 ± 0.4 (4-6)	3.3 ± 1.9 (0-6)	1.0 ± 1.0 (0-2)	<0.0001
IADL	7.1 ± 1.4 (3-8)	3.6 ± 2.5 (0-8)	1.6 ± 1.7 (0-5)	<0.0001
CIRS-CI	3.0 ± 1.6 (0-8)	4.0 ± 2.1 (1-9)	4.7 ± 2.3 (1-8)	0.0016
ESS	18.9 ± 1.5 (13-20)	15.6 ± 2.0 (12-20)	12.6 ± 2.8 (8-18)	<0.0001
SPMSQ	1.0 ± 1.4 (0-6)	2.0 ± 2.1 (0-9)	4.6 ± 2.6 (0-10)	<0.0001
Social Condition				0.0148
Living alone	19.8	29.2	43.8	
Living with family	78.1	70.8	43.8	
Institutionalized	2.1	0.0	12.5	
Number of Drugs				<0.0001
0-3 drugs	63.5	25.0	6.3	
4-6 drugs	32.3	45.8	37.5	
≥ 7 drugs	4.2	29.2	56.3	
MPI				
MPI	0.20 ± 0.08 (0.06-0.31)	0.50 ± 0.09 (0.38-0.64)	0.75 ± 0.07 (0.69-	-

Mortality				
6 months	16.7	56.3	75.0	<0.0001
12 months	29.2	70.8	81.3	<0.0001

Data are presented as % or mean±SD (min-max)
NS: not significant differences.

Table 2D: Univariate Hazard Risks of mortality, age- and sex-adjusted, according to the MPI, the individual factors used to calculate the MPI and other possible mortality predictors after 6 months and 12 months of follow-up

Predictors	**6 months**				**12 months**		
	HR	**95% CI**	**p-value**		**HR**	**95% CI**	***p*-value**
General and oncological characteristics							
Educational Level (years)	1.039	0.966-1.117	0.3076		1.051	0.989-1.118	0.1111
BSA (m^2)	0.062	0.014-0.277	0.0003		0.095	0.026-0.356	0.0005
CIRS-SI	4.804	2.681-8.608	<0.0001		3.984	2.358-6.732	<0.0001
MMSE	0.959	0.913-1.007	0.0944		0.981	0.938-1.025	0.3893
GDS	1.241	1.141-1.350	<0.0001		1.186	1.101-1.277	<0.0001
VNS	1.147	1.055-1.246	0.0013		1.142	1.063-1.227	0.0003
CBI	1.037	1.018-1.056	<0.0001		1.032	1.016-1.049	0.0001
Site of primitive tumor			<0.0001				0.0001
Lung	1.111	0.503-2.245	0.7955		1.057	0.545-2.168	0.8789
Stomach/Esophagus	1.098	0.492-2.499	0.8202		0.949	0.448-2.028	0.8924
Breast	0.308	0.123-0.768	0.0116		0.323	0.148-0.706	0.0046
Colon-rectal	0.170	0.068-0.425	0.0001		0.296	0.150-0.585	0.0005
Other sites	1				1		
Metastasis	1.934	1.059-3.530	0.0318		1.993	1.197-3.320	0.0080
Oncological Treatments							
Chemotherapy	1.468	0.857-2.514	0.1618		1.693	1.064-2.693	0.0262
Radiotherapy	1.568	0.760-3.236	0.2234		1.344	0.687-2.630	0.3879
Endocrine-therapy	0.431	0.131-1.416	0.1657		0.493	0.195-1.250	0.1364
Surgery	0.605	0.312-1.172	0.1361		0.572	0.324-1.008	0.0534
MPI Domains							
MNA	0.838	0.799-0.879	<0.0001		0.850	0.816-0.885	<0.0001
ADL	0.689	0.616-0.771	<0.0001		0.724	0.656-0.799	<0.0001
IADL	0.753	0.687-0.825	<0.0001		0.798	0.737-0.864	<0.0001
CIRS-CI	1.307	1.138-1.500	<0.0001		1.246	1.105-1.406	<0.0001
ESS	0.783	0.726-0.845	<0.0001		0.816	0.762-0.875	<0.0001
SPMSQ	1.082	0.957-1.224	0.2082		1.060	0.949-1.184	0.3010
Social Condition			0.7304				0.7133
Living with family	1				1		
Living alone	1.182	0.632-2.213	0.6003		1.246	0.732-2.121	0.4179
Institutionalized	1.632	0.385-6.912	0.5059		1.199	0.287-5.000	0.8036
Number of Medications			0.1126				0.1752
0-3 drugs	1				1		
4-6 drugs	1.395	0.762-2.553	0.2810		1.271	0.761-2.120	0.3593
≥ 7 drugs	2.143	1.044-4.399	0.0378		1.802	0.967-3.357	0.0638
MPI							
MPI			<0.0001				<0.0001
Low	1				1		
Moderate	4.358	2.269-8.270	<0.0001		3.565	2.1116-6.006	<0.0001
Severe	8.094	3.749-17.475	<0.0001		5.655	2.866-11.158	<0.0001
MPI*	3.595	2.492-5.188	<0.0001		2.937	2.142-4.027	<0.0001

*0.3 units increase in MPI

Table 2E: Multivariate Cox regression models age and sex adjusted for mortality after 6 months and 12 months of follow-up

Predictors	**6 months**				**12 months**		
	HR	**95% CI**	**p-value**		**HR**	**95% CI**	***p*-value**
MPI*	5.959	3.214-11.048	<0.0001		5.130	2.946-8.934	<0.0001
CIRS-SI	5.010	2.167-10.553	<0.0001		5.060	2.543-10.070	<0.0001
BSA	0.009	0.001-0.082	<0.0001		0.039	0.007-0.218	0.0002
GDS (≥5)	3.621	1.773-7.395	0.0004		2.605	1.501-4.519	0.0007
MMSE	1.126	1.043-1.215	0.0024		1.128	1.054-1.207	0.0005
Chemotherapy	3.201	1.662-6.168	0.0005		3.559	2.010-6.301	<0.0001
Site (lung)	3.456	1.681-7.104	0.0007		2.701	1.431-5.099	0.0022

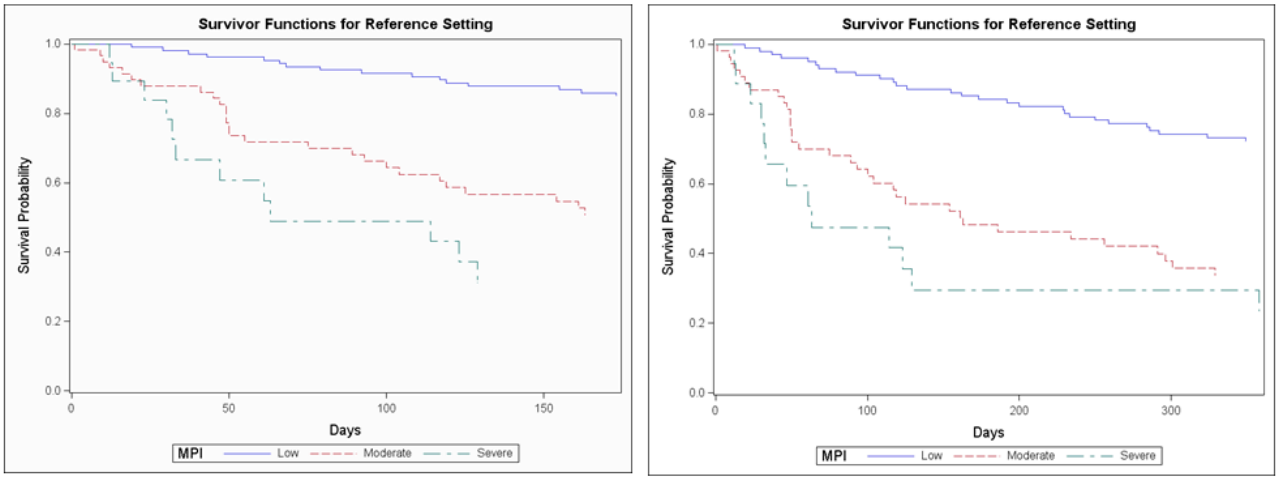
*0.3 units increase in MPI
0.1 units increase in MPI: HR=1.813, 95% CI=1.476-2.227 after 6 months, HR=1.725, 95% CI=1.434-2.075 after 12 months of follow-up
0.2 units increase in MPI: HR=3.287, 95% CI=2.178-4.961 after 6 months, HR=2.975, 95% CI=2.055-4.305 after 12 months of follow-up

Table 2F: Predictive performance of the MPI for 6 months and 12 months of overall survival

	6 months Survival				12 months Survival		
	AUC ROC	**95% CI**	***p*-value**		**AUC ROC**	**95% CI**	***p*-value**
Unadjusted model	0.815	0.746-0.884	<0.0001		0.778	0.706-0.849	<0.0001
Age adjusted model	0.817	0.748-0.886	<0.0001		0.780	0.709-0.852	<0.0001
Age and sex adjusted model	0.814	0.743-0.884	<0.0001		0.778	0.706-0.850	<0.0001
Age and sex adjusted model + other mortality predictors*	0.914	0.869-0.959	<0.0001		0.874	0.819-0.928	<0.0001

AUC ROC: area under the receiver operating characteristics curve
* CIRS-SI, BSA, GDS (≥5), MMSE, Chemotherapy Treatment, Site (lung)

Figure 2A: age and sex adjusted survival curves for different grades of MPI at 6-month (left) and 12-month (right).



CHAPTER 3

The Toxicity Risk

ESTIMATING THE RISK OF CHEMOTHERAPY TOXICITY IN VULNERABLE ELDERLY CANCER PATIENTS: THE ROLE OF VULNERABLE ELDERS SURVEY-13 (VES-13)

3.1 BACKGROUND AND AIMS

Comprehensive geriatric assessment (CGA) is a multidimensional tool used by geriatricians and oncologists to detect and evaluate multiple age-related problems of general health and all of the functional, cognitive, social and psychological parameters of older subjects, and to plan and coordinate appropriate interventions (Rubenstein, 1984). Such approach has already been shown that it can offer considerable benefits in various settings and medical conditions and, in oncology, CGA can provide clinical informations that are not revealed by performance status (Monfardini, 1996; Repetto, 2002; Luciani, 2012). Recent oncological studies have shown that some geriatric parameters are predictive of chemotherapy toxicity, and specific scores have been identified in order to help physicians in their everyday clinical practice (Extermann, 2012; Falandry, 2013; Hurria, 2010).

However, as CGA is time consuming and can rarely be used routinely by oncologists, some short screening instruments have been validated for elderly patients with cancer (Balducci, 2010). The most widely studied of these is the Vulnerable Elders Survey (VES-13) (Molina-Garrido, 2011; Owusu, 2011; Luciani, 2010; Mohile, 2007; Biganzoli, 2013; Pottel, 2012), a 13-item function-based scoring system that considers age, self-rated health, limitations in physical function and functional disabilities, and provides a simple and clinically relevant means of identifying older people with an increased degree of vulnerability (Min, 2006) ; its geriatric validation showed that vulnerable patients were at 4.2-fold greater risk of dying during the following two years (Saliba, 2001). In comparison with a full CGA, it has good sensitivity and acceptable specificity (Luciani, 2010), but there is not consistent medical literature regarding its ability to predict

chemotherapy toxicity. We therefore collected the clinical records of patients enrolled in four published clinical trials of VES-13 (Luciani, 2010; Biganzoli, 2013; castagneto, 2013; Falci, 2009) with the aim of evaluating its accuracy in predicting the risk of grade 3/4 toxicity in elderly patients undergoing chemotherapy.

3.2 PATIENTS AND METHODS

3.2.1 Patients

The study involved patients aged >70 years with a histologically or cytologically confirmed diagnosis of a solid or hematological tumor able to comprehend Italian language and no other active cancer diagnosis. All of the patients underwent a VES-13 evaluation by a trained physician before receiving cancer treatment.

The study was approved by the Ethics Committee of the coordinating centre.

3.2.2 Functional status

The patients with a VES-13 score of ≥3 were considered vulnerable; as VES-13 classifies all patients aged >85 years as vulnerable, these reach a score of 3 solely on the grounds of age. Performance status was expressed using Zubrod's 0-5 scale, with zero being the best, 4 the worst and 5 classifying death.

3.2.3 Comorbidities

Comorbidities were recorded as a total number and assessed using the Cumulative Illness Rating Scale for Geriatrics (CIRS-G), which rates 14 conditions on the basis of their severity (0 = no problem; 4 = severe or life threatening). The results are expressed as a

total score and a comorbidity index (total score divided by the number of affected organs and systems) (Miller, 1992). An index of >3 and/or a severity category of 3-4 was considered a sign of disability.

3.2.4 Medications

The number and type of medications (including those used for pain and nausea, as well as over-the-counter medications) were recorded at the time of the evaluation.

3.2.5 Cognitive and social status

Cognitive status was assessed using Folstein's Mini-Mental State Evaluation (MMSE). A score of <24 suggested a cognitive impairment.

The social status assessment included the patient's living conditions (alone, with a family member, with friends, or in an assisted residence), and the presence and adequacy of a designated caregiver.

3.2.6 Nutritional status

Nutritional status was preliminarily assessed by means of the Mini-Nutritional Assessment®. If the total score was <17, the patients were considered at risk of malnutrition (Gerber, 2003; Barone, 2003; Bleda, 2002).

3.2.7 Treatment

All of the patients were allowed to receive chemotherapy for early or advanced disease in accordance with the international guidelines for each specific cancer type. A record was

kept of the type of chemotherapy, the number of cycles, the number of dose reductions, the reason for each reduction, the number of treatment interruptions, and the patient's response. Toxicity was assessed using the Common Terminology Criteria for Adverse Events (CTCAE) v. 4.0, and grade 3-4 hematological and non-hematological toxicities were considered in the analysis.

3.2.8 Statistical analysis

Characteristics of the studied sample were summarized overall and by group defined by VES-13 score, using mean, standard deviation (SD) and minimum-maximum values for continuous variables, absolute and relative frequencies for categorical variables. Comparisons of baseline and tumor characteristics between vulnerable and non vulnerable groups were made by Chi-square test or Fisher test for categorical variables or Wilcoxon test for continuous ones. A logistic regression model was used for univariate and multivariate analyses to test demographic characteristics and clinical features for their association with the risk of grade 3-4 toxicity occurrence. Variables found to be possibly associated ($p<0.15$) or biologically relevant in the univariate model were considered for multivariate analysis. Results are expressed as odds ratios (ORs) with their 95% confidence intervals (95% CIs). Unless otherwise specified, statistical significance was set at $p=0.05$ for a bilateral test. Analyses were carried out with SAS Software, version 9.2 (SAS Institute, Cary, NC).

3.3 RESULTS

3.3.1 Patients

The study involved 648 Caucasian patients aged >70 years, whose baseline characteristics are shown in Table 3A. Their mean age was 76.2 (SD 4.5), ranging from 66 to 90 years, 336 (51.9%) were female, and all were capable of speaking and reading Italian language. Most (58.8%) of them were married and 91.0% had an adequate caregiver. They had a median number of two comorbidities (ranging from 0 to 9), and were taking a median number of three medications (ranging from 0 to 13). The most prevalent comorbidities affected the cardiovascular system, and the prevalent medication categories were antihypertensive and cardiovascular drugs. Lung as well as colorectal cancer each accounted for 26.1% of the patients; breast and ovarian cancer accounted for 8.6% and 9.7% of patients respectively.

3.3.2 VES-13 and treatment

VES-13 identified 287 of the patients (44.3%) as vulnerable: i.e. with a score of >3 (Tab. 3A). Their mean age was 77.6 (SD 4.9), ranging from 67 to 90 years; 57.1% were female with a median number of three comorbidities (ranging from 0 to 9). Their performance status and CIRS index were both worse than those of the non-vulnerable patients (Tab. 3B), and fewer received polychemotherapy (38.3% vs. 62.6%, $p < 0.001$). Most of the patients received first-line and adjuvant chemotherapy (Tab. 3B); only 1.4% received radiotherapy in a sequantial schedule.

3.3.3 Toxicity

As shown in Table 3C, grade 3-4 hematological and non-hematological toxicities were more prevalent in the vulnerable subjects (35.2% vs 20.8%, p <0.0001, and 18.5% vs 10.8%, p = 0.0055). A total of 120 vulnerable patients (41.8%) underwent dose reductions, which were due to toxicity in 61 cases (50.8%); 86 patients (30.0%) interrupted their treatment, mainly because of disease progression (44 patients, 51.2%).

3.3.4 VES-13 and toxicity

At univariate analysis VES-13 score, sex, age, propensity score, MMSE score and CIRS score resulted biologically relevant, possibly or significantly associated with grade 3-4 toxicity occurrence, therefore they were selected for multivariate assessment. In the multivariate model, vulnerable patients (OR 2.15, 95% CI 1.46-3.17; p<0.001) and females (OR 1.53, 95% CI 1.05-2.23; p=0.025) had a higher risk of hematological toxicity, whereas increase of CIRS appear to be protective (OR 0.90, 95%CI 0.83-0.97; p=0.008) (Table 3D).

The variables included in the multivariate analysis of non-hematological toxicity (Table 3D) were age, performance status, VES-13 score, and the presence of metastasis. The vulnerable patients had an OR of 1.66 (95% CI 1.02-2.72; p = 0.043) and those with a metastatic disease had an OR of 0.59 (95% CI 0.37-0.94; p = 0.026).

The Forest plot by age subgroup (Fig. 3A) showed an higher risk of toxicity with increasing class of age. For non-hematological toxicity (Fig. 3B) this trend is less clear and no effects heterogeneity was observed between age subgroups.

3.4 DISCUSSION

The aim of this study was to assess the role of VES-13 (a screening instrument that has been validated in geriatric patients and is recommended by the NCCN clinical practice guidelines for senior adult cancer subjects) in predicting the risk of chemotherapy toxicity in a sample of elderly cancer patients undergoing chemotherapy. The patients identified as vulnerable by the VES-13 had a statistically significant higher risk of developing both hematological (OR 2.15, 95%CI 1.46-3.17; $p<0.001$) and non-hematological toxicity (OR 1.66, 95%CI 1.02-2.72; $p=0.043$). These risk increases progressively with the aging of the population, particularly for haematological toxicity.

In clinical practice, medical oncologists do need to identify the elderly patients who are at higher risk of developing toxicity due to cancer treatment. In two pilot studies, Extermann et al. (Extermann, 2002; Extermann, 2004) developed and validated the MAX2 index as a a means of comparing the average risk of severe toxicity due to various chemotherapy regimens, and this was recently included in a larger trial in which a number of clinical and geriatric items constituted a scoring system for predicting treatment toxicity (the CRASH score) (Extermann, 2012). Another study of a large sample of elderly patients found that some geriatric dimensions (the CARG score) can predict toxicity (Hurria, 2011). Although methodologically different, these two studies showed for the first time that clinico-biological variables (including those based on a geriatric evaluation) can play an important role in the menagement of chemotherapy in elderly cancer patients.

VES-13 was developed as a rapid method of identifying age-related problems and vulnerable subjects (Saliba, 2001) and has been validated in oncology as a short

instrument of distinguishing patients who should undergo a full geriatric evaluation from those who do not need to do so (Luciani, 2010; Biganzoli 2013; Extermann 2007). However, our findings indicate an important new role of the VES-13 in the management of elderly cancer patients which, to the best of our knowledge, has been described in only one previous study (Stoke, 2012).

Although the VES-13 was not originally designed to answer specific oncological questions, it has hereafter demonstrated an high capacity to estimate the overall risk of toxicity in elderly patients, especially in patients over 75 years old. The importance of our findings is reinforced by the fact that we used the most widely studied and previously validated assessment instrument in geriatric oncology. However, one point should not be ruled out: the VES-13 is widely used for screening purposes in everyday clinical practice and allows a pre-assesment of patients that eventually require a full geriatric evaluation (Rodin, 2007). Whenever possible, we encourage the use of a CGA in accordance with the guidelines. However, this time-consuming evaluation is not practicable in most oncological centres and a brief assessment instrument such as the VES-13 could help physicans to optimise assessment times and choose the most appropriate treatment. Fifty-five percent of the patients in our series were classified as being non-vulnerable, which means that a full CGA is potentially not required by more than half of the patients attending oncological centres.

Performance status (PS) is usually a useful means of assessing the functional status of older patients and making treatment decisions, but we found that it did not play any significant role in the estimation of cancer treatment risk. However, as one possible

reason for this is that only 11% of our patients had a PS of 2-3, we do not believe that PS should be excluded from the assessment of elderly cancer patients.

A second interesting finding of our analysis is the significant and apparently protective impact of comorbidity scores (CIRS-G) on toxicity. Historically, comorbidities and functional status have been considered two different models of evaluation that have a parallel impact on a patient's clinical status, although some trials have shown that they should be evaluated independently even if they have a combined effect (Extermann, 1998). On the basis of our findings, we believe that CIRS-G is an instrument should be used with caution when assessing the potential risk of chemotherapy toxicity.

In conclusion, our data indicate that, together with other scores, the VES-13 can be used in the assessment and risk management of elderly cancer patients undergoing chemotherapy. Future studies should be planned with the aim of prospectively comparing the various instruments or scores currently used in geriatric oncology in order to determine which are most appropriate in clinical practice when considering specific aspects of patient management (e.g. toxicity, overall survival, active life expectancy, or the quality of life).

Table 3A. Demographic and clinical characteristics by VES -13 scores of eligible patients.

Variable	**VES-13 <3**	**VES-13 ≥3**	**Overall***	**P-value**
Number of patients - N (%)	361 (55.7)	287 (44.3)	648 (100)	0.004§
Age in years – Mean (SD)	75.1 (3.8)	77.6 (4.9)	76.2 (4.5)	<0.001#
Min-Max	66-84	67-90	66-90	
Female sex - N (%)	172 (47.6)	164 (57.1)	336 (51.9)	0.016§
Marital status - N (%)				0.003¤
Not married	25 (7.2)	11 (3.9)	36 (5.8)	
Married	230 (66.3)	138 (49.5)	368 (58.8)	
Divorced	5 (1.4)	3 (1.1)	8 (1.3)	
Widow/er	87 (25.1)	127 (45.5)	214 (34.2)	
Unknown	14	8	22	
Education - N (%)				0.792§
No education	3 (0.8)	4 (1.4)	7 (1.1)	
Primary	217 (60.3)	170 (59.2)	387 (59.8)	
Secondary	100 (27.8)	84 (29.3)	184 (28.4)	
College	26 (7.2)	22 (7.7)	48 (7.4)	
University	14 (3.9)	7 (2.4)	21 (3.2)	
Unknown	1	-	1	
Caregiver presence – N (%)	323 (89.5)	267 (93.0)	590 (91.0)	0.115§
Comorbidities – Median	2	3	2	<0.001#
Min-Max	0-8	0-9	0-9	
Medications – Median	3	4	3	<0.001#
Min-Max	0-12	0-13	0-13	
ECOG PS - N (%)				<0.001§
0-1	349 (96.7)	227 (79.4)	576 (89.0)	
2-3	12 (3.3)	59 (20.6)	71 (11.0)	
Unknown	-	1	1	
CIRS (Total) – Mean (SD)	3.6 (2.4)	3.8 (2.5)	3.7 (2.4)	0.426#
Min-Max	0-12	0-12	0-12	
CIRS (Index) – Mean (SD)	1.6 (0.6)	1.7 (0.5)	1.6 (0.5)	0.031#
Min-Max	0-3	0-2.7	0-3	
MNA- N (%)				<0.001§
0	307 (85.0)	206 (71.8)	513 (79.2)	
1	54 (15.0)	81 (28.2)	135 (20.8)	
MMSE – Mean (SD)	27.8 (2.2)	27.4 (2.8)	27.7 (2.5)	0.002#
Min-Max	17-30	16.3-52	16.3-52	

Legends of table 3A: PS=Performance Status; VES-13: Vulnerable Elders Survey.
***** The percentages in the column "Overall" are calculated having as denominator the total number of patients
[§] Chi-square Test
[#] Wilcoxon Rank-Sum Test
[¤] Fisher's Exact Test

Table 3B. Tumour and treatment characteristics by VES-13 in the eligible patients

Variable	VES-13 < 3	VES-13 ≥ 3	Overall*	P-value
Primary tumor site – N (%)				0.003§
Colon-rectum	110 (30.5)	59 (20.6)	169 (26.1)	
Endometrium	20 (5.5)	13 (4.5)	33 (5.1)	
Breast	36 (10.0)	20 (7.0)	56 (8.6)	
Ovary	23 (6.4)	40 (13.9)	63 (9.7)	
Pancreas	17 (4.7)	21 (7.3)	38 (5.9)	
Lung	88 (24.4)	81 (28.2)	169 (26.1)	
Other	67 (18.6)	53 (18.5)	120 (18.5)	
Metastasis– N (%)	238 (65.9)	218 (76.0)	456 (70.4)	0.006§
Chemotherapy – N (%)				< 0.001§
Monochemotherapy	135 (37.4)	177 (61.7)	312 (48.1)	
Polychemotherapy	226 (62.6)	110 (38.3)	336 (51.9)	
Response – N (%)				0.001§
CR	138 (38.2)	78 (27.2)	216 (33.3)	
PR	78 (21.6)	50 (17.4)	128 (19.8)	
NC	59 (16.3)	56 (19.5)	115 (17.7)	
PRO	86 (23.8)	103 (35.9)	189 (29.2)	
Treatment type - N (%)				0.311¤
CT	354 (98.1)	285 (99.3)	639 (98.6)	
CT+RT	7 (1.9)	2 (0.7)	9 (1.4)	
Chemotherapy type - N (%)				0.093¤
Neoadjuvant therapy	8 (2.2)	4 (1.4)	12 (1.9)	
Adjuvant therapy	123 (34.1)	68 (23.7)	191 (29.5)	
First line	220 (60.9)	213 (74.2)	433 (66.8)	
Second line	8 (2.2)	- (0.0)	8 (1.2)	
Third line	1 (0.3)	2 (0.7)	3 (0.5)	
Fifth line	1 (0.3)	- (0.0)	1 (0.2)	
Treatment type - N (%)				
Neoadjuvant therapy:				
- CT	7 (1.9)	4 (1.4)	11 (1.7)	
- CT+RT	1 (0.3)	- (0.0)	1 (0.2)	
Adjuvant therapy:				
- CT	118 (32.7)	66 (23.0)	184 (28.4)	
- CT+RT	5 (1.4)	2 (0.7)	7 (1.1)	

First line:				
- CT	219 (60.7)	213 (74.2)	432 (66.7)	
- CT+RT	1 (0.3)	- (0.0)	1 (0.2)	
Second line:				
- CT	8 (2.2)	- (0.0)	8 (1.2)	
Third line:				
- CT	1 (0.3)	2 (0.7)	3 (0.5)	
Fifth line:				
- CT	1 (0.3)	- (0.0)	1 (0.2)	

* The percentages in the column "Overall" are calculated having as denominator the total number of patients

§ Chi-square Test

¤ Fisher's Exact Test

Table 3C. Treatment compliance and toxicity

Variable	VES-13 < 3	VES-13 ≥ 3	Overall*	P-value**
Dose Reduction (First Cycle) - N (%)	50 (13.9)	37 (12.9)	87 (13.4)	0.722[§]
Dose Reduction - N (%)	131 (36.4)	120 (41.8)	251 (38.8)	0.160[§]
Unknown	1	-	1	
Cause of Dose Reduction – N (%)				0.788[¤]
Toxicity	70 (53.8)	61 (50.8)	131 (52.4)	
Acute event	1 (0.8)	- (0.0)	1 (0.4)	
Medical decision	42 (32.2)	41 (34.2)	83 (33.2)	
Subject decision	6 (4.6)	3 (2.5)	9 (3.6)	
Progression	10 (7.7)	14 (11.7)	24 (9.6)	
Death	1 (0.8)	1 (0.8)	2 (0.8)	
Unknown	1	-	1	
Treatment Interruption – N (%)	106 (29.4)	86 (30.0)	192 (29.6)	0.868[§]
Cause of Treatment Interruption – N (%)				0.025[¤]
Toxicity	33 (31.1)	23 (26.7)	56 (29.2)	
Acute event	1 (0.9)	2 (2.3)	3 (1.6)	
Medical decision	17 (16.0)	3 (3.5)	20 (10.4)	
Subject decision	16 (15.1)	12 (14.0)	28 (14.6)	
Progression	36 (34.0)	44 (51.2)	80 (41.7)	
Death	2 (1.9)	2 (2.3)	4 (2.1)	
Other	1 (0.9)	- (0.0)	1 (0.5)	
Haematological Toxicity (Grade 3-4) – N (%)	75 (20.8)	101 (35.2)	176 (27.2)	
Non Haematological Toxicity (Grade 3-4) – N (%)	39 (10.8)	53 (18.5)	92 (14.2)	

***** The percentages in the column "Overall" are calculated having as denominator the total number of patients
[§] Chi-square Test
[¤] Fisher's Exact Test

Table 3D. Effect of different parameters on hematological toxicity. Univariate and multivariate logistic regression models

	Haematological toxicity				Non haematological toxicity			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
Variable	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**
Female sex	1.81 (1.27-2.59)	0.001	1.53 (1.05-2.23)	0.025	1.18 (0.76-1.84)	0.458	Not included	
Age (for each 1-year increase)	0.99 (0.96-1.03)	0.762	0.97 (0.93-1.01)	0.189	1.05 (1.00-1.10)	0.052	1.03 (0.98-1.09)	0.184
Ves13 (for each 1-unit increase)	1.10 (1.04-1.18)	0.003	Not included		1.09 (1.01-1.17)	0.037	Not included	
Ves13 ≥ 3	2.07 (1.46-2.94)	<0.001	2.15 (1.46-3.17)	<0.001	1.87 (1.20-2.92)	0.006	1.66 (1.02-2.72)	0.043
Presence of metastasis	1.17 (0.80-1.72)	0.423	Not included		0.64 (0.41-1.02)	0.058	0.59 (0.37- 0.94)	0.026
ECOG PS								
0-1 (reference)	1		1		1			
2-3	1.90 (1.14-3.17)	0.014	1.37 (0.79-2.37)	0.270	2.10 (1.16-3.82)	0.015	1.64 (0.87-3.08)	0.126
CIRS (Total) (for each 1-unit increase)	0.89 (0.82-0.96)	0.003	0.90 (0.83-0.97)	0.008	0.95 (0.87-1.05)	0.320	Not included	
CIRS (Index) (for each 1-unit increase)	1.12 (0.80-1.56)	0.504	Not included		0.76 (0.51-1.13)	0.171	Not included	
MNA positive	1.07 (0.70-1.63)	0.772	Not included		0.99 (0.57-1.70)	0.963	Not included	
MMSE (for each 1-unit increase)	1.06 (0.99-1.14)	0.123	1.08 (1.00-1.17)	0.043	1.05 (0.96-1.14)	0.325	Not included	
Response								
CR+PR (reference)	1				1			
NC+PRO	0.89 (0.63-1.27)	0.528	Not included		0.99 (0.64-1.54)	0.971	Not included	

Figure 3A. Forest plot of ORs (VES-13 ≥3 *vs* VES-13 <3) of hematological toxicity by age group

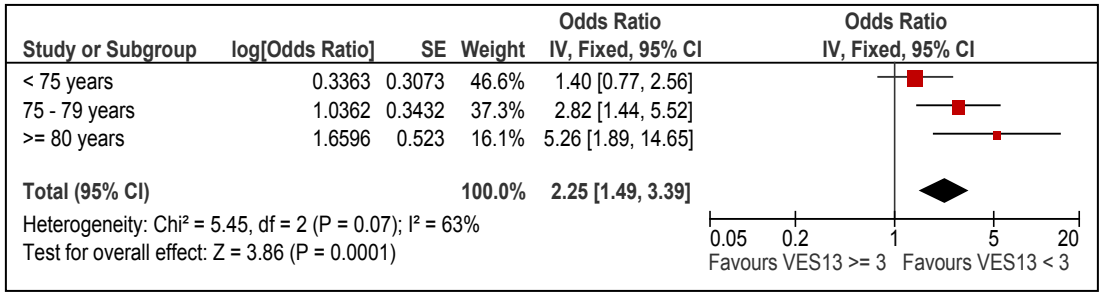
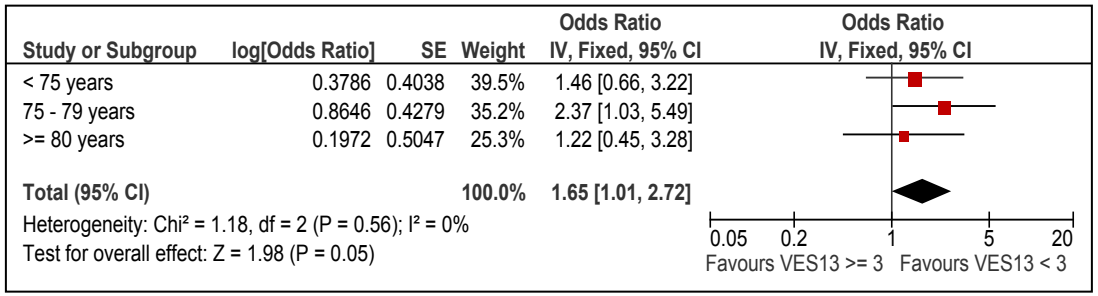


Figure 3B. Forest plot of ORs (VES-13 ≥3 *vs* VES-13 <3) of non-hematological toxicity by age group



CONCLUSIONS

EXPLORING THE RELATIONSHIP BETWEEN AGEING AND CANCER: FROM TRANSLATIONAL TO CLINICAL RESEARCH

The Research Activity, described in the present thesis, has been devoted to shed light into some critical aspects of Geriatric Oncology.

Despite cancers in the elderly represents an important epidemiological issue, for decades cancer patients aged 70 years or more have been excluded from clinical trials and from the relevant progresses in anticancer treatments that became available to younger adults.

The undertreatment of elderly cancer patients, due to underestimation of survival benefits and overestimation of toxicity risks, is still a relevant problem as well as the fact that less than 10% of cancer centres in Italy have a special unit dedicated to the management of elderly patients. Certainly, a better knowledge of the relationship between ageing and cancer is essential to optimize the cancer treatment in aged patients and both translational and clinical research should be performed to accelerate the progresses in Geriatric Oncology.

The first study reported in the present thesis was the first to approach the problem of immunesenescence in elderly cancer patients. The study provided the first evidence that elderly cancer patients seemed to suffer from a more severe decline in thymic output and had a lower proportion of naïve $CD8^+$ cells than age-matched controls. In addition, cancer patients had significantly shorter telomeres in their peripheral blood cells than age-matched non-cancer patients. This result suggests the unpublished hypothesis – which would need to be tested in a larger study - that elderly people with shorter telomeres are at higher risk of developing cancer. It is noteworthy that the cancer patients' thymic output decreased significantly with increasing age, while telomere length in the peripheral blood cells was unassociated with age. Finally, this preliminary study also confirms that there is no relationship between the geriatric phenotypes investigated by the CGA and these molecular markers of aging.

Really, in the lack of molecular markers that estimate the biological age of single individuals, the CGA remain the only way of estimating the life-expectancy and the chemotherapy toxicity. Therefore, the second chapter of this thesis reported a prospective trials that was the first to validate in the oncological setting a standardized version of CGA to be used in further clinical trials, as

required by the International Society of Geriatric Oncology. In the present study the MPI retained in elderly patients with advanced cancer the same reliability and accuracy as reported in the original study by Pilotto et al. The results also suggested the possibility of creating a new, better-performing version of MPI by integrating it with the comorbidity severity index and the geriatric depression scale.

Finally, the third challenge of the Geriatric Oncology is to validate a fast version of CGA that may be easily applied to elderly cancer patients in order to estimate the risk of chemotherapy toxicity before any treatment decision. For this purpose the Vulnerable Elders Survey – 13 by Mohile-Saliba seems to be the best option. The ability of VES-13 to predict chemotherapy toxicity in elderly cancer patients was tested in 4 italian, prospective trials that have been joined in a combined analysis. The patients identified as vulnerable by the VES-13 had a statistically significant higher risk of developing both hematological and non-hematological toxicity. These risk increases progressively with the aging of the population, particularly for haematological toxicity.

In conclusion, the present thesis provided two important evidences:

1. The physiology of ageing in cancer patients can be profitably studied by translational research, in order to suggest possible biological markers of cancer risk, cancer mortality or toxicity, to optimize the approach to cancer treatment in elderly patients. In this case, markers of immunesenescence and telomere length deserve to be tested in larger cohorts. If confirmed, they could be widely used in elderly general population to easily identify subjects who run an higher risk of developing cancer and optimize the resources for screening procedures.

2. MPI seems to be the right candidate for the standardized geriatric assessment in the context of clinical trials, where specific geriatric endpoints are never currently used, as well as VES-13 correctly estimates the risk of chemotherapy toxicity. With the awareness that geriatric assessment of cancer patients cannot relies on a single test, future studies should be planned with the aim of prospectively identifying which is the most appropriate geriatric instrument for any single aspect of

patient management (e.g. toxicity, overall survival, active life expectancy, or the quality of life) and clinical research.

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