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Contents

Volume 23 / n. 1 March 2018

Reviews

Research

Case reports

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Correlation between the autonomic nervous system and neoplastic disease

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Summary. The physiological role of the autonomic nervous system (ANS) includes maintenance of homeostasis and response to stressors. Sympathetic nervous system (SNS) is involved at early stages of tumorigenesis via β-adrenergic signaling and via central and local norepinephrine/epinephrine release from SNS nerve fibers. Parasympathetic nervous system (PNS) promotes invasion and dissemination of cancers using cholinergic receptors. In this paper, we review published evidence regarding the impact of SNS and PNS on solid tumor development, and discuss the importance of those findings for clinic and prevention.

Key words: autonomic nervous system, sympathetic nervous system, parasympathetic nervous system, neoplasm

Physiological function of the autonomic nervous system

Autonomic nervous system (ANS) consists of two main parts: sympathetic nervous system (SNS, noradrenergic) and parasympathetic nervous system (PNS, cholinergic). Both of them play a significant role in maintaining homeostasis and stress response. Physiological mechanisms involved in the autonomic activity include neural outflow, synthesis, release and degradation of transmitters, ganglionic regulation and receptor-mediated effects.

Sympathetic fibers participate in many physiological processes, inter alia regulating efferent sympathetic nerve outflow and generating its differential patterns, synchronizing neural activity of various target tissues and organs and thus, controlling function thereof. Moreover, acting via β-adrenergic signaling pathway, SNS induces 'fight-or-flight' stress response. SNS fibers innervate all major organs, releasing catecholamine neurotransmitters (norepinephrine and epinephrine) in response to a physiological threat to homeostasis or acute sympathetic activation. Norepinephrine and epinephrine, the metabolites of amino acid, tyrosine, are released from the adrenal medulla during a stress reaction. Their biological effects include an increase in blood pressure, stimulation of liver glycogenolysis and airway dilation.

Parasympathetic nervous system provides innervation to many organs, controlling a number of vital physiological functions, such as heart rate, endocrine activity, digestion, gastrointestinal motility, inflammation and immune response. Vagus nerve, the X cranial nerve, and its branches contain 80% of afferent sensory fibers and 20% of efferent motor fibers, and form a communicating sensory pathway between the central nervous system and peripheral tissues. The main parasympathetic neurotransmitter is acetylcholine, acting via five types of muscarinic receptors (chrm1 – chrm5) and two types of nicotinic receptors (muscle-type, N1, and neuronal-type, N2).

Autonomic contribution to neoplastic processes – Underlying mechanisms

Tumor progression and dissemination depend on intrinsic prosperities of cancer cells, such as self-

renewal and the ability to migrate (invasiveness). Tumor stroma, containing fibroblasts, endothelial cells and immune cells, may interact with cancer cells (1, 2). This microenvironment regulates growth of a primary tumor and formation of metastases. Both stromal cells of the tumor and signaling molecules interact directly and indirectly with the nervous system (3). The metastatic cascade involves two main steps. During the first stage, tumor cells migrate from the primary tumor to the site of hematogenous and lymphogenous dissemination. During the second stage, the cells extravasate from the circulation and invade surrounding tissues (4, 5).

Both sympathetic and parasympathetic components of the autonomic nervous system play vital roles during the development and spread of solid tumors, albeit at different stages of tumorigenesis. Likewise in leukocyte and fibroblast migration, neurotransmitters regulate also the migratory activity of cancer cells. Cancer cells may migrate along nerve fibers; this phenomenon, referred to as perineural invasion, is associated with poorer prognosis (6-10). SNS mediates tumor initiation and progression through a variety mechanisms. β-adrenergic signaling promotes the inhibition of DNA damage repair and p53-associated apoptosis via several molecular pathways (e.g. β-arrestin-induced activation of the AKT signaling pathway); as a result, SNS may contribute to tumor initiation and/or chromosomal instability (1, 2). Macrophages play an important role, modulating tumor microenvironment and promoting metastasis. β-adrenergic signaling stimulates recruitment of macrophages to tumor parenchyma via chemotactic factors, such as macrophage colony stimulating factor (CSF1). Moreover, β-adrenergic signaling contributes to an increase in tumor-associated macrophage density, promoting myelopoietic development of monocyte precursors in the spleen and bone marrow. Finally, β-adrenergic signaling stimulates macrophage expression of gene programs that initiate tumor progression within the tumor microenvironment.

Additionally, β-adrenergic signaling may also modulate various growth and survival pathways, among them programmed cell death mediated by focal adhesion kinase (FAK) (11). SNS generally promotes tumor progression through an array of pleiotropic molecular alterations in the microenvironment of primary tumor (12). Tumor cells may spread via lymphatic and blood vessels, and along serous membranes (13). Perineural invasion results from interactions between cancer cells and nerve fiber microenvironment. Sympathetic nerves may contribute to the perineural invasion and stimulate tumor growth. Cancer cells stimulate the expression of stromal cell-derived factor 1 (also referred to as C-X-C motif chemokine ligand 12, CXCL 12) and insulin-like growth factor 1 (IGF-1) in stromal cells; this eventually contributes to the selective growth of cancer cells clones that are hypersensitive to these factors and primed for spread in CXCL12- and IGF-1-enriched bone marrow (14) (Fig. 1). This pathway is also implicated in spread of cancer cells in patients who do not show the evidence of blood and/or lymphatic metastases (15, 16). A reciprocal signaling interaction between tumor cells and nerves contributes to peripheral nerve invasion. Neurotrophic factors (NGF) and axonal guidance molecules are pivotal for axonal growth (17, 18). However, these molecules and their receptors are also localized in tumor cells and hence, the latter can bind to the neurites (19, 20). According to Liebig et al., peripheral nerve invasion can be diagnosed whenever tumor cells are present within any of the three layers of the nerve sheath (epineurium, perineurium, endoneurium), or if tumor foci exist outside the nerve, involving at least 33% of its circumference (16). Also ANS may interfere with angiogenesis and modulate tumor microenvironment. Cancer cells can release neurotropic factors, such as axon guidance molecules, which stimulate the growth of nerve fibers, blood vessels and lymphatics (neoangiogenesis and lymphangiogenesis, respectively) supplying the tumor (9, 21, 22).

Moreover, neoplastic cells can release angiogenic factors, such as vascular endothelial growth factor (VEGF), and angiogenic chemokines, which also promote neoangiogenesis (21, 23). Tumors with diameters greater than 1 cm cannot be effectively supplied with nutrients without the development of new blood vessels (neoangiogenesis) (21). NGF, acting alone or in combination with 6-hydroxy-dopamine (6-OHDA), was shown to stimulate neoangiogenesis in the superior cervical ganglia of newborn rats; similar effect was also observed in breast and prostate cancers, whereby

NGF initiated neoangiogenesis and apoptosis, and modulated the severity cancer-induced bone pain (24-26). VEGF is a mitogen for endothelial cells, and promotes angiogenesis in vivo. Both VEGF and its receptors (expressed on sympathetic nerve fibers innervating arteries) promote vascular sympathetic innervation. VEGF promotes sympathetic axon growth. Its effects on vascular sympathetic innervation are modulated by other vascular-derived neuronal growth factors. Inhibition of VEGF was shown to prevent reinnervation in vivo (27). Aside from angiogenesis, VEGF was also shown to modulate lymphangiogenesis; since it shows a neurotrophic activity within the peripheral nervous system, this factor may also contribute to neurogenesis (23, 28-30).

Survival and growth of cancer cells may be also promoted by nerve-derived growth factors released from nerve fibers located in close vicinity of the tumor (31, 32) (Fig. 1). Fibroblast growth factor (FGF) regulates synaptogenesis and neuronal migration and therefore, may also contribute to the development of tumor innervation (33). Aside from the stimulation of hematopoiesis, granulocyte-colony stimulating factor (G-CSF), a hematopoietic stem cell mobilizer with established clinical application, may also modulate neuronal activity. This was confirmed in a mouse study, in which G-CSF affected survival of autonomic nerve fibers, which resulted in growth and dissemination of prostate cancer cells (34). Secretion of soluble factors from prostate cancer cells may contribute to nerve

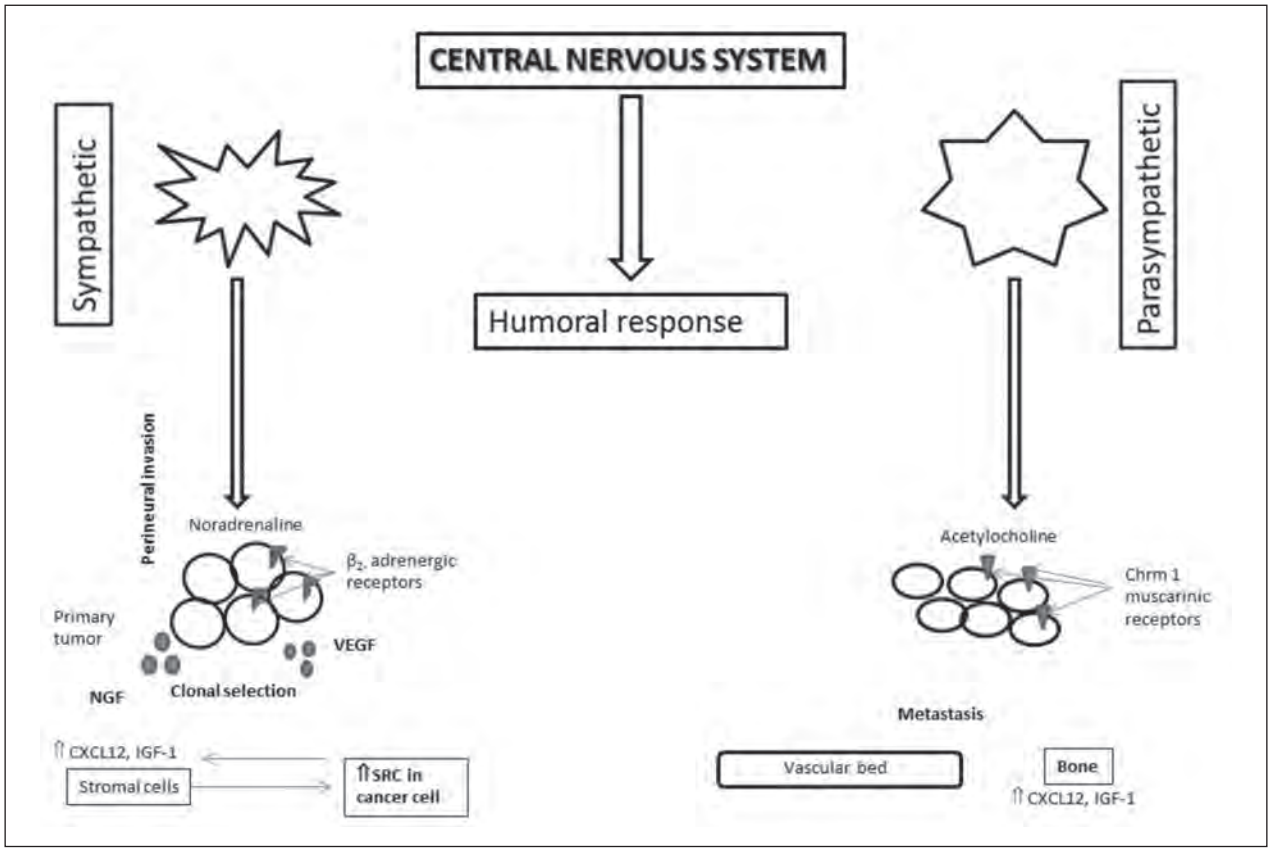

Figure 1. The role of sympathetic and parasympathetic regulation in cancer progression and dissemination. Sympathetic nerves contribute to perineural invasion of cancer cells, and stimulate tumor growth. Cancer cells induce the expression of CXCL12 and IGF-1 in stromal cells, which promotes selective growth of clones that are hypersensitive to these factors and primed for metastasis in CXCL12- and IGF-1-enriched bone marrow. In turn, parasympathetic nerves play a role in cancer cell expansion and dissemination (according to del Toro et al., modified) (14).

sprouting and/or branching (10). Due to presence of neuro-neoplastic synapses and expression of receptors for neural markers, cancer cells may effectively communicate with neurons. Neurotransmitters reach cancer cells via neuro-neoplastic synapses, stimulating their migration and therefore, contributing to tumor spread (35). Neuro-neoplastic synapses are functional units, rather than morphological entities (8). Neurotransmitters may stimulate the release of acetylcholine from a subset of CD4(+) T cells and thus, indirectly activate other immune cells, for example via upregulation of α7-nicotinic acetylcholine receptors on macrophages (36).

Autonomic nervous system and immunological processes

Both sympathetic and parasympathetic nervous system participate in neuroimmune processes. Immune cells (especially lymphocytes) express adrenergic and muscarinic receptors, as well as the receptors for acetylcholine (ACh), choline acetyltransferase (ChAT) and acetylcholinesterase (AchE). Neurotransmitters released from the sympathetic and parasympathetic nerve endings bind to their receptors and initiate immunomodulatory response. The interactions between SNS and immune system involve signaling mechanisms that provide a link between neurotransmitters and neuromodulators, co-transmitters (ATP and adenosine), adrenergic receptors and, albeit not necessarily, immune cells, cytokines and bacteria. SNS innervation of lymphoid tissue (the spleen and lymph nodes) modulates the evolution of peripheral immune response via cytokines, and promotes humoral immune responses at an expense of cellular immunity (12). Neuronal and neuroendocrine pathways are involved in communication between the nervous and immune system. Vagus nerve has been implicated as a component of the neural pathway transmitting signals from the peripheral immune system to the brain. Also cutaneous sensory afferents have been postulated to provide a communication pathway to central neural circuits. The immune to CNS communication is mediated by three non-neuronal mechanisms: cytokine transport system, brain structures containing blood vessels with fenestrated capillaries, and some molecules (e.g. prostaglandins, cytokines) that may reach the brain parenchyma (37). Hence, the neuronal system may contribute to cancer spread via the immune system (38). Chronic stress and depression may predispose to tumor spread due to impairment of immune response associated with decreased number of cytotoxic T-cells and natural killer (NK) cells (39).

Cholinergic anti-inflammatory pathway may involve cholinergic-adrenergic interactions at peripheral sites, autonomic ganglia and lymphoid targets (37, 40-43). The synthesis of immune and inflammatory mediators (cytokines, chemokines and free radicals) and the activity of various lymphoid cells are modulated due to activation of adrenergic receptors (37, 40-41). The activation of adrenergic receptors may affect synthesis of many compounds, among them tumor necrosis factor-alpha (TNFα), interleukins (IL-6, IL-10 and IL-12), chemokine macrophage inflammatory protein 1 alpha and a free radical, nitric oxide (41).

Analysis of genomic profiles identified specific patterns related to various disease entities. For example, one previous study revealed commonalities in a genetic signature, which turned out to be associated with both β-adrenergic receptor and cancer (3). Breast cancer and β-adrenergic pathway were demonstrated to share some genetic signatures, namely IL-6, MMP9, MMPI, FOSB, LCK, ERG, CCL2I, RHOJ, IGFI and ETSI; this observation supports the hypothesis on a link between β_2-adrenergic receptor and breast cancer pathway, and may constitute a foundation for new anticancer therapies based on adrenergic receptor strategies (44). β-adrenergic pathway may influence oncogene pathways, such as *Her2* and SRC (45-46). Indeed, β-adrenergic pathway has already been shown to stimulate the phosphorylation of SRC by protein kinase A, which resulted in SRC-mediated activation of a complex phosphoproteomic network promoting tumor growth and invasion in vivo (45). Furthermore, a positive correlation between β_2-AR level (β_2-adrenergic receptor) and *Her2* status was reported in breast cancer cells. Activation of β-adrenergic receptor by a catecholamine contributed to the upregulation of *Her2* mRNA expression and to the stimulation of signal transducer and activator of transcription 3 (STAT3); the latter molecule activates ERRB promoter to stimulate gene transcription (47). Furthermore, β-adrenergic signaling

pathway may prevent DNA repair and suppress p53 level and t53-associated apoptosis via molecular pathways, such as Rad3-related (ART)/p21 pathway (1-2, 48-49). While this mechanism was already demonstrated to be sufficient for an increase in the number of spontaneous chromosomal aberrations in the tissues, we still do not know whether the β-adrenergic inhibition of DNA repair may contribute to the initiation of spontaneous tumorigenesis in vivo (12).

Sympathetic Nervous System (SNS)

The effects of catecholamine neurotransmitters are mediated by receptors from α_1, α_2, β_1 β_2 and β_3 families. β-adrenergic receptors are G-protein coupled receptors that activate adenylate cyclase to "synthesize" intracellular 3',5'-cyclic adenosine monophosphate (cAMP); the latter activates protein kinase A (PKA) to phosphorylate serine or threonine in target proteins. PKA is involved in regulation of cellular metabolism, growth, differentiation, secretion, motility, neurotransmission and gene transcription. The second cAMP effector is guanine nucleotide Exchange Protein by Adenylyl Cyclase (EPAC). EPAC signaling may alter cellular morphology, motility and secretion (3). β-adrenergic signaling pathway regulates activity of various cells, among them epithelial cells, vascular myocytes, myeloid and lymphoid immune cells (50). All these cells express adrenergic receptors and therefore, are prone to the autonomic control. β-adrenergic signaling contributes to migration of cancer cells (51-53) and regulates VEGF-dependent angiogenesis (54-56) (Fig. 1). Also matrix metalloproteinase-related enhanced tissue invasion remains under the β-adrenergic control (56-58). Additionally, proapoptotic protein BAD (B-cell lymphoma 2-associated death promoter) may contribute to the development of chemotherapy resistance, acting via β-adrenergic receptor (11). β-adrenergic receptors, primarily β_2, are expressed at metastatic sites: in the brain, lungs, liver, lymphoid tissue, etc. (54, 59-60). β-adrenergic signaling pathway modulates the migratory potential and invasiveness of cancer cells via alterations in tumor gene expression and via upregulation of matrix metalloproteinases (MMP-6 and MMP-9) (57). Moreover, adrenergic receptors may modulate pro-metastatic consequences of tumor immune response. Activation of adrenergic receptors initiates infiltration of tumor tissues by macrophages and promotes a pro-metastatic gene expression signature. A consequence of macrophage infiltration is overexpression of macrophage-derived factors (e.g. COX2, MMP-9, VEGF) (61-62). Due to the activation of beta$_2$-adrenoreceptor-cAMP-protein kinase A pathway, epinephrine and norepinephrine inhibit secretion of type 1 proinflammatory cytokines: interleukin-12 (IL-12), TNFα and interferon-γ by antigen-presenting cells and Th1 helper cells. Furthermore, these endogenous catecholamines promote secretion of type 2 anti-inflammatory cytokines: interleukin-10 (IL-10) and transforming growth factor β (TGFβ) (40). These neurotransmitters can suppress Th1 response, which results in the inhibition of cell-mediated immunity and predominance of humoral immune response. Activation of SNS during the course of immune response may limit the inflammatory reaction, promoting accumulation of neutrophils and stimulating more specific humoral response; this protects the host against unfavorable effects of proinflammatory cytokines and other compounds released from activated macrophages (40). SNS is involved at early stages of tumorigenesis via β-adrenergic signaling, and systemic and local release of norepinephrine/epinephrine from sympathetic nerve fibers (3, 6, 9, 63-65). This results in an increase the number of axons and promotes ramification thereof. Furthermore, cancer cells can produce axon guidance molecules and neurotrophic factors, such as NGF (66-67). The axon guidance molecules contribute to the development of new blood vessels. Four families of the guidance molecules exist: netrins, slits, ephrins and semaphorins. Netrins are bond by UNC5 and deleted in colorectal cancer (DCC) receptors, whereas slits, ephrins and semaphorins interact with slits-roundabout receptors (Robos), Ephrin receptors (Eph) and plexins/neuropilins, respectively (68). While netrins are known to prevent cancer cell apoptosis and thus, participate in tumorigenesis, their role in cancer cell migration is yet to be established (69). Semaphorins play an important regulatory role in carcinogenesis (70). For example, SEMA3B and SEMA3F act as tumor suppressors (71), and overexpression of SEMA3B was demon-

strated to cause apoptosis in breast and lung cancer cell lines (72). Ephrins and their receptors are involved in carcinogenesis through diverse mechanisms; for example, EphA4 receptor was shown to induce proliferation and migration of glioblastoma cells (73). Moreover, both ephrins and their receptors are the regulators of tumor microenvironment, providing a link between cancer cells and surrounding stroma (74).

In turn, vascular growth factors, e.g. VEGF, were shown to control the development of sympathetic innervation (75). Sympathetic axons follow the newly developed arteries that release neurotrophic factors, such as endothelin and artemin (76-77). However, this relationship seems to be bidirectional, since also sympathetic nervous system was demonstrated to provide some regulatory input to angiogenesis and arteriogenesis (78-79). These complex interactions explain tumor's potential to develop its own neurovascular network. SNS may influence the neoplastic growth, modulating tumor microenvironment in both primary and metastatic target sites, via β-adrenergic regulation of myelopoiesis (80-82). β-adrenergic pathway influences expression of genes involved in oncogenesis and tumor spread, inter alia those controlling inflammatory processes, neoangiogenesis, cellular immune response and programmed cell death (12, 83). Furthermore, β-adrenergic signaling pathway promotes development of hematological malignancies, interfering with stem cell biology and physiological hematopoiesis (12).

Stress is associated with the release of IL-6, a proinflammatory cytokine playing pivotal role in cancer spread. Stress stimulates the sympathetic-adrenal-medullary axis to release catecholamine hormones, such as norepinephrine (9). The latter may be involved in upregulation of metalloproteinases (MMP-2, MMP-9) and VEGR; due to their modulatory effects, the proinflammatory cytokines may inter alia contribute to greater invasiveness of nasopharyngeal carcinomas (56). Moreover, stress induces norepinephrine and β-adrenergic receptors under IL-6 regulation (56, 84). Aside from being a key inflammatory mediator, transcription factor NF-κB may also contribute to carcinogenesis and tumor spread. One direct transcriptional target for NF-κB is neuronal guidance molecule, netrin-1, which was shown to be upregulated during the course of inflammatory processes.

β-adrenergic receptors were found in the breast, prostate and pancreatic cancer cells as well as in melanoma cells; their activation by catecholamine neurotransmitters resulted in enhanced activity of those cells (9, 85-88).

Parasympathetic Nervous System (PNS)

Parasympathetic nervous system promotes invasion and dissemination of cancer cells, acting via cholinergic receptor muscarinic 1 (chrm 1) expressed in tumor stroma (6, 7, 63, 89). The activation of chrm1 by acetylcholine results in cancer spread. This phenomenon has been first demonstrated in a mouse cancer model, and then confirmed in patients with prostatic adenocarcinoma (6-7, 87, 89). According to Espanol et al., the expression of muscarinic acetylcholine receptor in murine mammary adenocarcinoma cells may modulate their aggressiveness (90). However, published data on the role of vagal innervation in the development of rat fibrosarcoma are inconclusive, and we still do not know if formation of metastases could be controlled with an inhibitor of the PNS signaling pathway (91). Nevertheless, Magnon et al. confirmed that autonomic nervous system plays a role in the development and spread of human prostate cancer (7). Tumor cholinergic signals are mediated by stromal chrm1 expression. Cholinergic fibres of PNS play role in tumor cell invasion, migration and distant metastases (7).

PNS plays an important role in the inflammatory reflex. Inflammation is a driving force for the dissemination and thus, inflammatory pathways and their effector cells, both controlled by the nervous system, contribute substantially to cancer spread. Inflammatory signals generated within the bowel may significantly alter peripheral neuronal signaling, which results in both peripheral and central sensitization, a phenomenon that is reflected by an enhanced afferent neuronal activation (92). As shown recently, the brain not only can "detect" peripheral inflammation via afferent vagal fibers, but may also attenuate innate immune activation due to an integrated neural response involving massive activation of vagal efferent fibers. This efferent arm of the inflammatory reflex is referred to as the "cholinergic anti-inflammatory pathway". The inflammatory reflex is a key

system involved in maintenance of homeostasis. Vagus nerve, the arc of the reflex and neural-related factors, such as netrin-1 and neuropeptides, all participate in the inflammation control (93, 94). Moreover, activation of vagus nerve exerts an effect on the production of cytokines by leukocytes. The electrostimulation of the vagus results in a decrease in cytokine production in the spleen, and an opposite effect has been observed after surgical ablation of this nerve (93). The cholinergic anti-inflammatory pathway requires signaling from the nicotinic acetylcholine receptor subunit α7, which inhibits the splenic nerve to suppress cytokine release by splenic macrophages (95).

Vagus nerve can influence immune cell function in the spleen through preganglionic and postganglionic system of neurons. The preganglionic system originates from the dorsal motor nucleus of the vagus, and the postganglionic system, from the ganglia of the celiac superior mesenteric plexus (94).

Role of non-cholinergic and non-adrenergic projections in carcinogenesis

Dopamine produced in the brain is a catecholamine. However, unlike for norepinephrine and epinephrine, only few reports describe the role of dopamine in tumor cell migration and metastasis (52, 96-97). Dopamine participates in regulating gene expression, such as induced tumor cell migration. It acts through dopamine receptors (DRs) activation which are members of seven transmembrane domain trimeric guanosine 5'-triphosphate (GTP)-binding protein-coupled receptor family (97). DRD2/DARPP-32 expression is associated with tumor progression, therefore DRD2/DARPP-32 expressions can be a predictive factor (97).

Chemokines bind to receptors from the G-protein-coupled receptor (GPCR) family to regulate tumor-associated angiogenesis and tumor-specific immune response of the host, and to stimulate tumor cell proliferation in an autocrine manner (98). These findings may be the potential target of the therapy for cancer patients.

Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter of the brain and an inhibitor of glucagon secretion in the pancreatic α cells. Involvement of GABA receptors was demonstrated to block the migratory effect of norepinephrine in mammary, pancreatic and colon carcinoma (52, 99-100).

Substance P acts as a neurotransmitter, neuromodulator and inflammatory mediator. However, this molecule can be also involved in the development of bone marrow metastases of breast cancer and neuroblastoma (101). Moreover, substance P was shown to stimulate growth of basal-like human breast carcinoma cell line, MDA-MB-468 (52).

Taken altogether, those findings suggest that GABA-receptor agonists and the blockade of substance P receptor (NK-1) may constitute novel strategies in anticancer therapy.

ANS and Heart Rate Variability

In a clinical setting, the autonomic activity can be assessed based on Heart Rate Variability (HRV) analysis. Monitoring of HRV is a non-invasive method to examine autonomic innervation of the heart and the vegetative modulation of the sinus node. The time- and frequency-domain analysis of the HRV provides an information on the autonomic balance. Furthermore, HRV is suitable for the quantification of sympathetic and parasympathetic tone (102). HRV analysis is a routine, widely-available method to evaluate vagal activity based on several indices, such as standard deviation of all normal beat to beat intervals (SDNN), root mean square successive difference between adjacent normal beat to beat intervals (RMSSD), the number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording divided by the total number of all NN intervals (pNN50), and high frequency (0.15-0.4 Hz) component (HF). Low values of the HRV analysis parameters may reflect sympathetic predominance or a decrease in parasympathetic input (102).

Vagal tone, expressed by the HRV, may constitute a new independent prognostic factor in patients with some solid tumors (103-105). Enhanced vagal activity may slow down tumorigenesis and produce a protective effect in patients with advanced or/and metastatic cancers (106). Several studies demonstrated a positive

association between high-frequency HRV component and overall survival in cancer patients; this relationship was independent of potential confounders, such as anticancer treatment, patient age and sex (106).

Previous studies demonstrated that prognosis in cancer is inter alia determined by the parasympathetic activity. De Couck et al. evaluated ANS in subjects with colorectal, pancreatic, prostate, lung and ovarian malignancies; the study demonstrated that cancer patients presented with significantly lower values of HRV parameters (SDNN and RMSSD) than healthy controls (103). Furthermore, the values of those HRV indices turned out to be significantly lower in patients with advanced cancers than in individuals with early malignancies. Other factors, such as age and sex, did not exert a significant effect on HRV analysis parameters in the study groups (103).

Another study revealed that vagal activity may be a prognostic factor in cancer patients. Patients diagnosed with colorectal and prostate cancers presented with higher levels of carcinoembryonic antigen (CEA) at 12 months and prostate-specific antigen (PSA) at 6 months, respectively, whenever their values of HRV indices were low (104). Furthermore, a positive correlation was found between vagal activity and overall survival in patients with advanced pancreatic cancer. According to Mouton et al., vagal activity may play a role in cancer recurrence (105). As widely known, the level of CEA, a cancer marker, correlates with the risk of relapse in most colorectal cancer patients. However, the study revealed that 12 months after anticancer treatment, patients who had increased vagal tone at the baseline (SDNN>20 ms) presented with lower levels of CEA than individuals with lesser HRV (SDNN<20 ms). Moreover, higher baseline HRV turned out to be associated with longer overall survival, also after adjustment for age and anticancer treatment (106). Moreover, high-frequency HRV component, a measure of parasympathetic overactivity, was shown to correlate positively with time to death in patients with terminal hepatocellular carcinoma and non-lung cancer (107, 108).

These findings may explain why prognosis in some cancer patients is worse than in the others; this phenomenon may be related to autonomic dysregulation of cardiac function. A dysfunction of ANS in cancer patients, especially sympathovagal disturbances, may contribute to changes in cardiovascular system regulation. Lower values of HRV indices (markers of vagal activity) are associated with increased risk for life-threatening arrhythmias, which is reflected by generally worse prognosis.

ANS and cancer-induced fatigue

Cancer-related fatigue has been long time ago recognized as a multi-factorial problem present in cancer patients. The issue has been inter alia addressed in a trial including breast cancer survivors who had completed anti-cancer treatment within past two years. Compared to their less-fatigued counterparts, the subjects who reported more fatigue had lower parameters of HRV analysis (only RMSSD was evalated) and presented with significantly higher norepinephrine levels prior to and after the exposure to the study stressor. Those findings point to a potential link between a decrease in parasympathetic activity and greater fatigue. The level of fatigue was associated neither with the type of anticancer treatment nor with the type and stage of the malignancy. However, female patients who reported more fatigue were significantly older and showed age-specific changes in HRV; this implies that cancer-related fatigue may increase with age (109). A link between a decrease in selected HRV indices (indicators of parasympathetic activity) and greater fatigue in breast cancer patients has been also reported by Croswell et al. (110). In the study conducted by those authors, lower values of two HRV indices, RMSSD and HF**,** correlated not only with greater fatigue, but also with older age, higher values of body mass index (BMI) and higher concentrations of IL-6 and C-reactive protein (CRP). However, the relationship between HRV and fatigue did not seem to be modulated by inflammatory mediators (110).

Importance of Autonomic Malfunction for Clinical Practice and ……

Epinephrine and norepinephrine act via β-adrenergic receptor signaling pathway. Both preclin-

ical and in vitro studies demonstrated that adrenergic activation modulates apoptosis, promotes angiogenesis and other cancer hallmarks; all these effects can be abrogated by β-blockers. Therefore, treatment with β-adrenergic antagonists (e.g. β-blockers used widely in cardiology) may be a new therapeutic strategy to control of tumor progression (4, 111-112). There are three categories of beta-blockers. First generation β-blockers, i.e. non-selective β-blockers, such as propranolol, are antagonists of both β_1 and β_2 adrenergic receptors. Second generation β-blockers (e.g. atenolol) show higher affinity for β_1- than for β_2-adrenergic receptors. Third generation β-blockers (e.g. nebivolol) exert vasodilatory effects (113). Previous studies examined the effects of β-blockers in some malignancies, such as breast cancer, melanoma, pancreatic cancer and oral squamous cell cancer (62, 85, 114, 115). Pharmacological inhibition of β-adrenergic receptors resulted in downregulation of VEGF, and promoted apoptosis in cancer cells (54, 116). This implies that β-adrenergic receptor pathways may constitute a target for anticancer therapies; this application of β-blockers has been already tested in patients with various malignancies. In a retrospective study of patients with triple negative cancer, conducted by Melhem-Bertrandt et al., administration of β-blockers concomitant to neoadjuvant chemotherapy was associated with prolonged relapse-free survival (RFS), but not overall survival (OS) (117). In another study, women taking propranolol or atenolol have been diagnosed with less advanced breast cancers that those who did not receive β-blockers (118). This observation is consistent with the results of a previous study in which β_2-adrenergic signaling pathway blockers inhibited progression of breast cancer. In the latter study, women who have been treated with β-blockers prior to breast cancer diagnosis, had a 57% lesser risk of tumor spread and a 71% lower cancer-specific 10-year mortality rate than other study subjects (119). Administration of β-blockers during the course of platinum-based chemotherapy was also identified as an independent positive prognostic factor in epithelial ovarian cancer patients after a cytoreductive surgery. Women from β-blocker group had longer median progression-free survival and median overall cancer-specific survival than other study subjects, in 27 vs. 17 months and 56 vs. 48 months, respectively (120). Also in a retrospective cohort study of patients with non-small-cell lung cancer, administration of β-blockers turned out to be associated with improved metastasis-free survival, disease-free survival and overall survival (121).

Another study conducted in Denmark demonstrated that administration of β-blockers was associated with a lower mortality risk in melanoma patients (122). However, another study did not document a significant effect of β-blockers, propranolol or atenolol, on colorectal cancer-specific mortality (123). Also, in a Norwegian cohort study of prostate cancer patients, administration of β-blockers was not associated with cancer-specific and overall mortality, PSA level, Gleason score and tumor stage at diagnosis. However, the same study demonstrated a significant decrease in cancer-specific mortality in a subgroup of men who received a β-blocker together with androgen deprivation therapy (ADT) (124). Administration of β-blockers did not contribute to significant differences in median progression-free survival and overall survival in patients with recurrent platinum-sensitive ovarian cancer, also after adjustment for age, platinum-based therapy-free interval, study treatment and performance status (125).

Despite numerous preclinical and clinical studies, we still need more evidence to confirm those findings. The results of some previous studies are inconclusive. Furthermore, we still do not know the optimal timing and duration of β-blocker treatment. At least 36 clinical studies analyzing the applicability of β-blockers in anticancer treatment are either currently ongoing or have just been completed, and we still wait for publication of their results.

…. and Prevention

Functioning of the neurotransmitter pathways may be affected by many extrinsic factors, such as lifestyle, exercise, physical activity, diet, concomitant diseases (e.g. cardiovascular disorders, diabetes mellitus), stress, unfavorable psychosocial work conditions and pharmacotherapy (126-128). Nicotinic acetylcholine receptors regulate the stimulation and inhibition of neurotransmitters and consequently, control the syn-

thesis and release of growth factors, angiogenic factors and neurotrophic factors in cancer cells, tumor microenvironment and metastatic foci. Some lifestyle-related factors (e.g. smoking) may upregulate nicotinic acetylcholine receptors and thus, contribute to stimulation of cancer cells (90). Therefore, healthy lifestyle with adequate level of physical activity seems to be crucial not only for cardiovascular prevention but also for the reduction of cancer risk.

Conclusions

Knowledge of interactions between cancer cells and ANS seems to be a key for the development of novel anticancer therapies. However, to develop such tailored anticancer treatments, we need more information about the role of sympathetic and parasympathetic pathways in various malignancies.

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High-risk follicular lymphoma patients: identification and treatment

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Summary. Over the last 15 years, the outcome of patients with follicular lymphoma (FL) has dramatically improved mainly as a result of effective therapies and of a better understanding of lymphoma biology. Although progression-free survival is approximately 10 years with standard treatment and overall survival upwards of 20 years, the clinical behavior among individual patients is highly heterogenous, and a significant number of subjects have a higher and earlier risk of dying from FL within a few years from diagnosis. In this article, we provide an overview of available prognostic tools that can be used to identify high-risk patients with FL and describe which therapies are available and can be recommended for this group of hard-to-treat FL patients.

Key words: follicular lymphoma, prognosis, prognostic factors, high risk, early progression

Introduction

Follicular Lymphoma (FL) is the most frequent subtype among indolent B-cell Non Hodgkin Lymphomas (NHL), typically diagnosed during the 5th to 6th decades (1). Over the last 15 years, the outcome of FL patients has dramatically improved mainly as a result of effective therapies and of a better understanding of lymphoma biology. Standard treatment for patients with advanced stage disease requires the combination of chemotherapy with anti CD20 immunotherapy. R-CHOP or R-bendamustine regimens are alternative options, with similar anti-lymphoma activity and with a different toxicity profile; they can be followed by rituximab maintenance, which allows excellent disease control that translates into a median progression-free survival (PFS) of approximately 10 years and overall survival (OS) upwards of 20 years (2-5). With the use of the novel antiCD20 monoclonal antibody obinutuzumab instead of rituximab, further improvement in patient survival is foreseen (6). Although most patients with follicular lymphoma follow an indolent course, the clinical behavior among individual patients is highly heterogenous, and a significant number of subjects are diagnosed with a hard-to-treat disease, with high risk of dying from FL within a few years from diagnosis.

Among known prognostic factors, duration of response has been recognized as a relevant driver of patient outcome in most lymphoma subtypes for many years now , but the impact of early progression has been well characterized in FL patients treated with standard immunochemotherapy only recently (7). Casulo et al. analyzed 588 FL patients from the National LymphoCare Study who received first-line rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). They identified 19% of cases with early progression of disease within 24 months after diagnosis (POD24), who had a five-year OS of 50%; this was significantly lower compared to the 90% observed for patients without POD24. This trend was maintained after adjustment for FL International Prognostic Index, and the results were validated in an independent set of 147 patients with FL who received first-line R-

CHOP. POD24 results were received with great interest by the lymphoma community as, for the first time in many years, one prognostic parameter was identified in a significant proportion of cases and with a clinically relevant effect on OS. Of note, POD24 was recently validated as a robust indicator of poor FL survival in a pooled analysis of >5.000 patients with FL included in 13 prospective clinical trials (8). Finally, in a subanalysis of the Gallium trial, the risk of POD24 was reduced by 34% in the obinutuzumab arm but its role as a bad prognostic factor for OS was confirmed (9).

POD24 is an important step towards the goal of personalized care for patients with FL but it also defines new, important questions that should be addressed. The two main questions concern the earlier identification of high-risk patients and which treatment should be offered to these patients in an attempt to overcome the bad outcome associated with early progression. Answering these two questions is a high priority. In this review article, we provide an overview of available prognostic tools that can be used to identify high-risk patients with FL and describe which therapies are available and can be recommended for this group of hard-to-treat FL patients. For the purposes of this article, the discussion will be limited to the risk of progression or of death and will thus not consider the risk of transformation of FL into an aggressive lymphoma. Transformation, however, should always be suspected at each relapse and, if possible, ruled out by confirming FL histology with a new biopsy.

Prognostic factors and prognostic scores in FL

Prognostic studies in FL can be classified into two main groups: those based on baseline features and those based on post-treatment assessment. A third group of studies then combines baseline and post-induction prognostics.

Baseline prognostic studies

Different approaches have been identified that use baseline clinical, biologic, or metabolic features to improve our ability to predict the natural history of FL in the individual subject. These include Follicular Lymphoma International Prognostic Index (FLIPI),

Table 1. Summary of prognostic factors used to identify high-risk patients and correlation with POD24

Score/factor	HR def.	HR%	Time	PFS (%)	OS (%)	POD24% in HR	Ref.
Baseline							
FLIPI	3-5 RF	28	5yrs	-	53	55	(10)
FLIPI2	3-5 RF	27	5yrs	29	59	-	(12)
TMTV	>510 cm^3	29	5yrs	33	85	56	(13)
m7FLIPI	calculated	28	5yrs	38 (FFS)	42-65	76	(14, 15)
23 gene model	calculated	21-35	5yrs	26	-	38	(16)
Post-induction							
EOI PET	DS 4-5	17	4yrs	23	-	87	(19)
MR t(14;18)	> 0e-4 DNA copies @12months	20-50	3yrs	41	-	-	(25)
Combined models							
TMTV + FLIPI2	>510 cm^3 and 3-5 RF	14	5yrs	46	87		(13)
FDG-PET + MR	DS 4-5 or > 10e-4 DNA Copies @12months	32	5yrs	35	-	-	(28)
TMTV + EOI PET	>510 cm^3 and DS4-5	8	5yrs	23	-	39	(29)

Table legend: HR: high risk; RF: risk factors; PFS: progression-free survival; FFS: failure-free survival; OS: overall survival; POD24: progression of disease within 24 months from treatment start; FLIPI: follicular lymphoma international prognostic index; TMTV: total metabolic tumor volume; EOI PET: end of induction PET; MR: molecular response, DS: Deauville score

FLIPI-2, baseline study of the Total Metabolic Tumor Volume (TMTV) with FDG-PET, and the definition of biological indexes, namely m7FLIPI and the 23-gene predictor score.

FLIPI and FLIPI2

The FLIPI and FLIPI2 scores are widely used risk models to predict the risk of death and of disease progression; they are both easy to calculate as they are designed to use simple clinical and laboratory features. FLIPI was developed thanks to extensive international cooperation in retrospectively collecting data of patients with FL diagnosed between 1985 and 1992 (10). The score is based on five prognostic factors (age, stage, LDH, number of nodal areas, and hemoglobin level) and was originally developed to predict OS, though none of the evaluated patients was treated with immunochemotherapy. High-risk patients were originally identified by FLIPI as those with 3 to 5 risk factors, accounting for 27% of cases, with these patients showing a 5-year OS rate of 52.5%. The index was subsequently validated for patients treated with standard R-CHOP and for PFS instead of OS (11). Of note, in the first description of POD24, FLIPI was also included in the multivariate analysis of overall survival, but only 55% of early progression was classified in the high-risk group (7).

FLIPI2 was developed by the same international consortium but was the result of prospectively collecting data of FL patients consecutively diagnosed, half of whom were also treated with conventional immunochemotherapy. FLIPI2 was based on the combination of 5 risk factors (age, bone marrow infiltration, hemoglobin level, beta2-microglobulin, longest diameter of largest lymph node), with high-risk patients having 3 to 5 risk factors. Similar to FLIPI, FLIPI2 identified 27% of patients as being at high risk; their 5-year PFS rate was 18.8% (12). Of note, no data are available to correlate FLIPI2 with the risk of early progression or POD24.

TMTV

The prognostic value of quantitative parameters obtained from baseline PET/CT has been recently reported in patients with various subtypes of lymphoma Among them, standardized measurement of the TMTV has shown particular usefulness. In a recent study by Meignan et al., baseline TMTV as a dichotomized variable was the strongest pre-treatment predictor of outcome in high tumor burden follicular lymphoma. The 29% of patients who had a high TMTV>510 cm3 had a markedly inferior 5-year PFS, with a median PFS of less than 3 years and an increased risk of death. Conversely, a metabolic volume below this cutoff in the remaining 71% of patients predicted a median PFS beyond 6 years. Importantly, TMTV was a strong predictor of early progression within the first 1-2 years after commencing therapy. Unlike the original FLIPI, FLIPI2 was also an independent predictor of PFS in this study and the combination of TMTV> 510 cm3 with intermediate-high risk FLIPI2 stratified the population into three risk categories based on the presence or absence of any of these two adverse factors. Of the 14% of patients with both a high TMTV and intermediate-high risk FLIPI2, 46% had a very poor 2-year PFS and 86% a 2-year OS. With a median progression-free survival of only 19 months, this population can no longer be characterized as having an indolent course (13).

A measure of the total burden of viable tumor and environmental cells offers a promising improvement on existing surrogates for tumor burden integrated into the current five-factor prognostic indices, FLIPI and FLIPI2. While the decision to treat follicular lymphoma is highly influenced by tumor burden, no specific study has ever addressed the prognostic role of the TMTV in FL and its added value to these clinical prognostic indices, which fail to adequately identify patients at particularly high risk of progression and early death after modern immunochemotherapy approaches.

m7-FLIPI

A first attempt to integrate clinical prognostic factors with biomarker analysis in the era of immunochemotherapy was made by Pastore et al., who integrated the mutational status of seven genes in the context of the FLIPI clinical backbone in a population of 151 high tumor burden FL patients who were treated

with standard R-CHOP. They used DNA deep sequencing to retrospectively analyze the mutation status of 74 genes and identified mutations associated with shorter failure-free survival in EP300, FOXO1 CARD11, and CREBBP genes, and mutations in EZH2, MEF2B, and ARID1A that were associated with longer failure-free survival. The model, called m7-FLIPI, was then calculated as the weighted sum of predictor values and included high risk FLIPI, poor ECOG performance status, and non-silent mutations in the above-mentioned genes. The m7-FLIPI identified a high-risk group of 28% of cases with a 5-year failure-free survival of 38% and a low-risk group with a 5-year failure-free survival of 77% (p<0.0001). The score outperformed FLIPI alone and FLIPI combined with ECOG performance status, and results were confirmed on an independent validation series (14). M7-FLIPI was also tested with POD24 in a different study, which used two independent series of patients with FL (GLSG 151 pts; BCCA 71 pts) and which showed that m7-FLIPI had the highest accuracy to predict POD24 (76% and 77%, respectively, in the two series). High-risk m7-FLIPI patients were significantly more likely to develop POD24, with an odds ratio (OR) of 5.82 (P=.00031) and 4.76 (P=.0052) in GLSG and BCCA patients, respectively. Compared with the FLIPI, the specificity of the m7-FLIPI in identifying POD24 (i.e., the true negative rate) in the two studies increased from 56% to 79% and from 58% to 86%, respectively (15).

23-gene predictor

An effort similar to m7-FLIPI to improve prognostication of FL patients using biomarker analysis was recently published by the LYSA group, which used a gene-expression profiling approach. The study was based on the gene expression analysis of 160 untreated high tumor burden FL patients enrolled in the phase III randomized PRIMA trial, with results validated using three independent international patient cohorts from LYSA, University of Iowa/Mayo Clinic Lymphoma SPORE, and the Barcelona Hospital Clinic. The study selected the expression levels of 23 out of 395 genes that were associated with a risk of progression to build a predictive model that identified a population at an increased risk of progression. This panel included genes previously described to be involved in B-cell development (VPREB1, FOXO1, FCRL2, AFF3, TCF4), apoptosis, DNA damage response (RASSF6, GADD45A), (E2F5, USP44), cell migration (CXCR4, SEMA4B, EML6, DCAF12, VCL, RGS10), immune regulation (CXCR4, KIAA0040, TAGAP, ORAI2, KIAA0040, METRNL), and other processes (PRDM15, ABCB1, ALDH2, SHISA8). In a multivariate Cox model for progression-free survival adjusted on rituximab maintenance treatment and FLIPI, this score independently predicted progression with an HR of the high-risk group compared with the low-risk group of 3.68 (P=<0.001). The high-risk group accounted for 21% to 35% of patients in the different series, and the 5-year PFS for the training set was 26% (95% CI 16-43) in the high-risk group and 73% (64-83) in the low-risk group. These results were confirmed in each validation group and in a combined validation cohort. In a multivariate analysis, the score predicted progression-free survival independently of anti-CD20 maintenance treatment and of the FLIPI score. In the combined validation cohort, the proportion of patients with POD24 was 19% (95% CI 15-24%) in patients with a low predictor score (low-risk group) but 38% (29-46%) in patients with a high predictor score (high-risk group), showing the model's ability to identify early relapse. Finally, the score was not prognostic for OS (16).

Both m7-FLIPI and the 23-gene model represent an important methodological step forward in the prognostic assessment of patients with FL and in the definition of high-risk patients. However, they both show important limitations mainly due to the difficulty in reproducing results and they both still lack clinical validation in the context of prospective studies and in different subgroups of FL patients (i.e., low tumor burden cases and patients treated with novel drugs).

Post-induction prognostic factors

Since radiology assessment was first used to define response to therapy in FL, the quality of response has rarely been identified as prognostic for PFS or OS (17).

Recently, response to therapy assessed either with FDG PET or with highly sensitive molecular techniques targeting the t(14;18) chromosomal translocation (minimal residual disease – MRD) have been suggested as important prognostic tools and have both been identified as pivotal factors in achieving the goal of personalized treatment.

Metabolic response

18-F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) has been identified as a strong diagnostic and prognostic tool in patients with Hodgkin lymphoma, diffuse large B-cell lymphoma, primary mediastinal B-cell lymphoma, and peripheral T-cell lymphoma. The prognostic role of metabolic response in FL patient was demonstrated in two large retrospective analyses of data from the PRIMA and from the FOLL05 trials and from one prospective trial by the LYSA group (18–20). In a pooled analysis of 246 patients from these three studies, Trotman et al. analyzed the application of centrally reviewed five-point Deauville scale (5PS) to assess the correlation between post-induction PET status and survival. Overall, 17% of patients were classified as non-responder using the Deauville score 4 (DS4) as cutoff to define PET positivity based on a higher concordance rate among independent reviewers (vs DS3). Interestingly, no significant correlation between most baseline characteristics and post-induction PET status was noted, apart from the grouped Ann Arbor stage, FLIPI score, grouped FLIPI score, and hemoglobin levels. The HR was 3.9 ($p<0.0001$) for PFS for patients with a positive PET scan versus those with a negative PET scan and was 6.7 ($p=0.0002$) for overall survival. Four-year PFS was 23% and 63% for patients with a positive or negative PET scan, respectively; four-year OS was 87% versus 97%, respectively ($p<0.0001$)(21). These data were robust enough to recommend the routine use of FDG-PET in FL patients as stated in the recently updated criteria for staging and response assessment in lymphomas (22, 23).

Actually, the prognostic strength of metabolic response in FL is supported by its stronger predictive role compared to FLIPI and FLIPI2 scores and by its ability to predict not only PFS but also OS. Available data provide a strong rationale to test the efficacy of response-adapted therapy in FL patients as well. Among possible limitations of the use of FDG-PET in lymphoma, we should acknowledge the low rate of high-risk patients, which is about 15% after R-CHOP immunochemotherapy, and the lack of full validation of the prognostic role of metabolic response with the use of bendamustine, of lenalidomide, and in the context of post-induction maintenance therapy with rituximab. New data on the prognostic role of metabolic response will be available with the final results of the randomized Gallium (R-chemio vs Obinutuzumab NCT01332968) and Relevance (R-Chemio vs. R-Lenalidomide NCT01476787) trials.

Molecular response

Most patients with FL achieve a complete response (CR) after treatment, but most of them will eventually relapse due to minimal residual disease (MRD).

The presence of t(14;18) chromosomal translocation and of clonal rearrangement of immunoglobulin genes in FL cells makes it feasible to use high-sensitivity techniques to detect the disease in peripheral blood and bone marrow sample and to work on the concept of molecular tumor burden and molecular response. Rambaldi et al. (24) assessed FL PCR through quantitative polymerase chain reaction (PCR) analysis for t(14;18) and IG gene rearrangement in a prospective study of 128 patients with FL treated with sequential CHOP and rituximab therapy. Molecular response (PCR negativity) was achieved in 32% of cases after CHOP and rose to 57% and 75% after rituximab and during follow up, respectively. For patients with a durable PCR-negative status, a better clinical outcome was also observed since freedom from recurrence was 57% (95% CI, 23-81) compared with 20% (95% CI, 4-46) in patients who never achieved or lost the molecular negativity ($P<.001$). In a second paper, Ladetto et al. studied the concept of molecular response in a randomized trial for untreated high-risk FL patients that compared standard CHOP-R with high dose therapy combined with rituximab (R-HDS). Molecu-

lar remission (MR) was achieved in 44% of CHOP-R and 80% of R-HDS patients (P<.001), and was the strongest independent outcome predictor, suggesting that achieving MR is critical to effective disease control, regardless of which treatment is used (25). More recently, Galimberti et al. analyzed the role of molecular tumor burden and response in patients enrolled in the randomized FOLL05 trial of immunochemotherapy for untreated patients with advanced stage FL.

At diagnosis, the molecular marker t(14;18) was detected in the bone marrow sample of 53% of cases. Patients without molecular marker or with a low molecular tumor burden (<1 × 10–4 copies) showed higher complete remission rate and longer PFS. Moreover, PFS was significantly conditioned by the PCR status at 12 and 24 months, with 3-year PFS of 66% for MRD– cases versus 41% for those MRD+ at 12 months (P=0.015), and 84% versus 50%, respectively, at 24 months (P=0.014) (26).

Based on these data, MR is confirmed as a promising prognostic factor in the post-induction assessment of response, as it is in other lymphomas or hematologic malignancies. The use of MRD in clinical practice, however, is limited due to the lack of consensus and standardization on MRD techniques and timing and to the lack of a molecular marker in all patients with FL; the rate of patients without a measurable marker is around 30%, which can only partially be improved with better methods and technologies (VDJ region analysis or rarer breakpoint regions of BCL2/IGH chromosomal translocation). Over the last few years, the concept that tumor cells undergoing apoptosis or necrosis release cell-free circulating DNA (cfDNA) into the blood has enabled the use of whole exome sequencing ("next-generation sequencing technologies" – NGS) to detect tumor presence from blood samples. Recently, Roschewski et al. used this technology to monitor response in 126 patients with diffuse large B-cell lymphoma; they showed that the presence of detectable cfDNA during surveillance was associated with a higher risk of lymphoma progression compared with that of patients with undetectable circulating tumor DNA (27). This new tool, called liquid biopsy, and the use of peripheral blood might further improve MRD studies in FL.

Combined models

All previously discussed prognostic factors were defined using multivariable models that also included commonly used clinical prognostic indexes of individual factors (13, 14). This suggests that prognostication of FL patients could be improved by combining different parameters as well as by integrating baseline and post-induction factors.

PET response and MRD

Luminari et al. combined metabolic and molecular response in a small group of 41 patients with FL for whom both MRD analysis and central review of post-induction PET were available. PET/MRD concordance was 76%, with Kappa=0.249, suggesting that PET and MRD when done at the end of induction therapy are not strongly correlated. Taken separately, the positivity rates were 27% and 11% for MRD and PET, respectively. In a stratified analysis combining the information on PET and MRD into 2 groups (PET–/MRD– vs. PET+ or MRD+), the achievement of both PET and MRD negativity (32% of cases) was associated to a better outcome, with a 5-yr PFS of 75% and 35% for PET/MRD –/– and PET+ or MRD+, respectively. Although conducted on a small series of patients, this study shows that combining EOT PET and MRD in patients with FL may improve our ability to predict the risk of progression (28). Based on these preliminary results, the Fondazione Italiana Linfomi designed the FOLL12 trial to investigate the efficacy of a response-adapted strategy in patients with FL (ClinicalTrials.gov Identifier: NCT02063685). This multicenter phase III randomized study has recently completed the enrollment of the planned 800 cases with newly diagnosed FLIPI2 intermediate-high risk stage II-IV FL requiring therapeutic intervention; subjects have been randomly assigned to either standard or experimental response-driven treatment (Figure 1). After a common induction treatment consisting of 6 cycles of R-CHOP or R-bendamustine, followed by 2 additional doses of rituximab, responding patients in the standard arm receive rituximab maintenance therapy (every 2 months for 2 years), while responding patients in the experimental arm are

assigned to different post-induction treatments based on PET and MRD results. PET- and MRD-negative patients undergo observation, PET-negative but MRD-positive patients receive pre-emptive rituximab therapy (4 weekly doses for a maximum of 3 courses until negativization of MRD), and PET-positive patients receive a consolidation (90)Y ibritumomab tiuxetan (0.4 mCi/kg) dose prior to starting conventional rituximab maintenance. This study aims to evaluate whether a PET and MRD response-based maintenance therapy is non inferior when compared to standard rituximab maintenance therapy in terms of PFS.

PET response and TMTV

Cottereau et al. combined metabolic response and TMTV in 159 patients with advanced stage FL from three prospective trials. In the univariate analysis, both high TMTV (>510 cm3) and positive EOI PET were independent, significant risk factors for PFS. Their combination stratified the population into three risk groups: 5-year PFS was 67%, 33%, and 23%, respectively, for patients without risk factors (64%), for those with one of the two adverse features (27%), and for patients with both adverse factors (8%); 10%, 39%, and 54%, respectively, were POD24. This model enhanced the prognostic value of PET staging and response assessment and allowed the identification of a small subset of patients with a very high risk of progression and POD24. (29)

Treatment of high-risk patients

Available guidelines for the treatment of FL patients do not recommend the use of prognostic factors

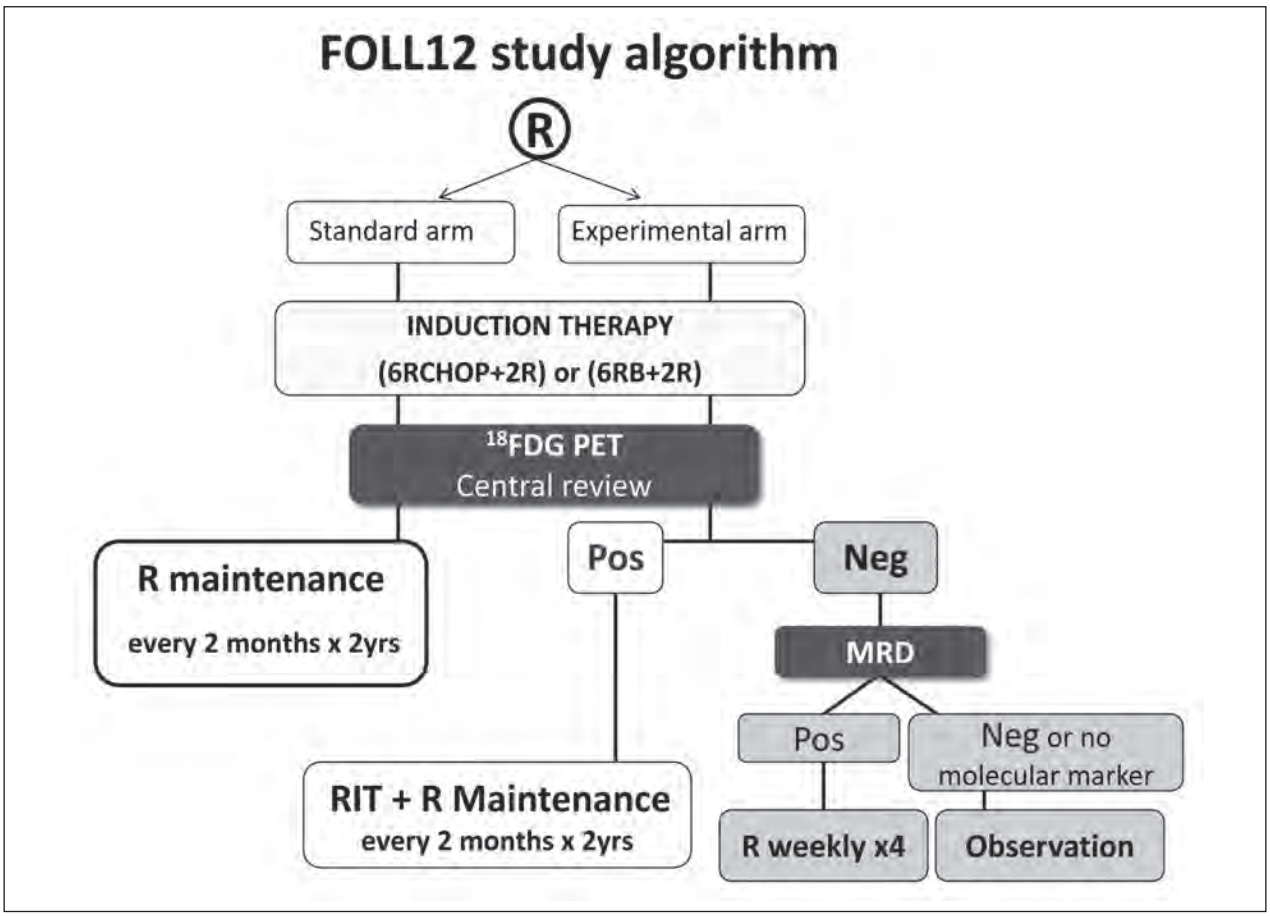

Figure 1. Design of the FOLL12 response-adapted trial for patients with stage II-IV high tumor burden follicular lymphoma.

to decide which treatment should be discussed with the patient. Clinical prognostic indexes are not considered decisional factors, and only stage, symptoms, and tumor burden (TB) are used to identify patients eligible for radiation treatment (stage I-II), immunotherapy or observation (stage II-IV with low TB), and immunochemotherapy (high TB). The same guidelines are extremely vague in defining recommendations for patients with relapsed refractory FL. In this setting, available options range from observation to the use of immunochemotherapy, the use of autologous stem cell transplant (ASCT), or one of the several new available drugs (30).

At first sight, then, no evidence is available to support any suggestions on how to treat high-risk patients with FL. There are, however, some data that can be used to recommend different therapies using validated definitions of high-risk patients. Moreover, clinical trials are starting to explore the concept of risk-adapted therapy in FL patients, as discussed above.

Among available options for relapsed refractory FL, there is a general consensus that ASCT should be used in FL patients who experience a relapse within 3 years from their first line of therapy and who are eligible for intensified treatment. The use of ASCT in relapsed refractory patients is supported by one positive but incomplete randomized trial and by a considerable number of retrospective studies, despite discordant results (31, 32).

The concept that ASCT could be effective in early relapsed patients suggests it is a good option for patients with POD24; unfortunately, in the original POD24 paper, it was not possible to assess the role of ASCT for patients with early relapse as only 8% of them actually followed the guidelines and were treated with ASCT as salvage therapy. Data to support the use of ASCT in early relapses can be found in two recent studies.

In the first, Casulo et al. analyzed data on 348 patients from the Center for International Blood and Marrow Transplant Research (CIBMTR) and the National LymphoCare Study (NLCS) to determine whether ASCT can improve outcomes in this high-risk FL subgroup. A first group of 174 patients with early failure who did not receive ASCT from NLCS was compared with a matched group of 175 patients who received ASCT obtained from CIBMTR. The planned subgroup analysis showed that patients receiving ASCT soon after treatment failure (≤1 year) had higher 5-year OS than those without ASCT (73% vs. 60%, P=.05). On multivariate analysis, early use of ASCT was associated with significantly reduced mortality (33). In the second study, Jurinovich et al. evaluated 113 patients with FL who were enrolled in 2 consecutive randomized trials of the German Low Grade Lymphoma Study Group who had POD24 and had not received prior ASCT. POD24 patients were more likely to receive ASCT as second-line treatment (46% vs 22%; p=0.008) compared to patients without POD24. In univariate and multivariate analyses, ASCT for POD24 patients was associated with significantly better 5-year second-line PFS and OS rates of 51% vs 19% and 77% vs 46%, respectively (34). In two additional retrospective studies, it was suggested that an allogeneic transplant in patients with POD24 could be more effective than ASCT (35, 36), but this option can only be offered to a small number of patients.

In summary, although based on retrospective studies, available data strongly support the hypothesis that standard conventional therapy for patients who are at high risk of POD24 is largely unsatisfactory and that if the patient is fit enough, the ASCT option should always be considered. Randomized trials comparing ASCT vs conventional immunochemotherapy for POD24 patients are strongly warranted.

Although several conventional therapies are available for patients who are not eligible for ASCT, few recommendations can be made, suggesting therefore the enrollment into a clinical trial as first option, if available, and using the most intensive treatment that can be tolerated by the patient as an alternative option. Some interesting data can be found on new drugs that have recently been approved by national health authorities for the treatment of relapsed refractory FL based on the drugs' activity as documented by phase II or phase III data. These include the pI3K inhibitor idelalisib, the immunomodulator lenalidomide, and the new anti CD20 monoclonal antibody obinutuzumab (37-39). Unfortunately, analysis for the subgroup of patients with early relapse are not available for either lenalidomide or obinutuzumab.

Idelalisib is an orally selective active phosphatidylinositol-3-kinase delta (PI3Kδ) inhibitor whose

activity was shown in a phase II study of 125 FL patients who had not had a response to rituximab and an alkylating agent or had had a relapse within 6 months (40). A retrospective post hoc analysis of the main study was conducted to examine whether idelalisib improved clinical outcomes in FL patients experiencing early progressive disease (PD) after initial chemoimmunotherapy. Of the 72 FL patients, 46 received first-line chemoimmunotherapy and 37 had early PD within ≤24 months from the start of treatment. The ORR was 21 out of 37 (57%), with 5 complete responses (14%) and 16 partial responses (43%). The median duration of response for all 37 patients with POD24 was 11.8 months (41). Interestingly, the efficacy and the safety results were not different between this subset analysis and the main study, suggesting that idelalisib can be considered a good option for the treatment of early relapsed patients who are not eligible for ASCT, or in some cases, as a bridge to ASCT.

Promising new drugs have recently started their clinical development, among them the EZH2 inhibitor tazemetostat (42), and checkpoint inhibitors have the best chance of moving ahead in their development(43).

Conclusions

In summary, several prognostic factors are currently available to identify a subgroup of approximately 30% of patients with FL whose lymphoma shows an aggressive clinical behavior and whose life expectancy is significantly reduced. Among available factors, POD24 has the strongest effect on outcome, but there is an urgent need to identify baseline features that can be used to define the prognostic profile earlier in the course of the disease. With highly active available immunochemotherapy regimens, a plateau in the curability of FL has probably been achieved, and a new generation of clinical trials should be started to test the efficacy of tailored treatment intensity to the individual risk of the patient. For the time being, treatment of high-risk FL should be based on available recommended options, including the use of ASCT and of new drugs when properly indicated.

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Acute Respiratory Distress Syndrome in cancer patients: epidemiology, risk factors and outcomes

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Summary. *Purpose:* This study aimed to evaluate the incidence and outcomes of Acute Respiratory Distress Syndrome (ARDS) in adult oncological patients in the ICU of a dedicated cancer hospital, as well as analyse the risk and protective factors associated with mortality in this population. *Methods:* A prospective cohort study evaluating all adult cancer patients admitted to the ICU, from January 2012 to December 2013. *Results:* The incidence of ARDS (n=87) was 11.9% of cancer patients in the ICU, and 17.8% among those in mechanical ventilation. ARDS was more common in onco-hematological patients. Patients with ARDS had longer ICU length of stay, more complications (mainly acute kidney injury [AKI]) and mortality than non-ARDS patients. Among patients with ARDS, those with a later ARDS onset (>48 h hospitalised) and with a more positive Fluid Balance (FB) had a higher mortality incidence. No differences were found in the ventilatory parameters, although the patients who died presented reduced pulmonary static compliance. *Conclusions:* The incidence and morbimortality of ARDS were high (particularly in onco-hematological patients). Later onset ARDS and highly positive FB presented a trend to a higher mortality.

Key words: Respiratory Distress Syndrome, adult, cancer, Intensive Care Unit, mortality

List of abbreviations

AKI	Acute Kidney Injury
APACHE:	Acute Physiology and Chronic Health Evaluation score
ARDS:	Acute Respiratory Distress Syndrome
COPD:	Chronic Obstructive Pulmonary Disease
Cstat	Pulmonary Static Complacency
FiO2	Fraction of inspired oxygen
ICU:	Intensive Care Unit
MV	Mechanical Ventilation
PaO2	Partial pressure of Oxygen
PEEP	Positive end-expiratory Pressure
PRBC	Packed Red Blood Cells
SD:	Standard deviation
TRALI	Transfusion-related Acute Lung Injury
VAP	Ventilation-associated Pneumonia

Introduction

Cancer remains one of the leading causes of death and hospital costs worldwide, particularly in developing countries (1, 2). The increase in survival rates, due to new screening and treatment strategies (3), also led to an increase in the incidence of admissions of cancer patients in the ICU (4). Oncological patients occupy up to 15% of all ICU beds with important medical, social and economic impacts (5-10).

Mortality caused by ARDS in cancer patients, particularly in onco-hematological malignancies, is superior to that of other ICU populations (11, 12) due to factors such as immunosuppression and infections, comorbidities, chemotherapeutic agents, radiation therapy, or the involvement of neoplastic tissue in the lung (13, 14).

*Objective***s**

This study aimed to evaluate the incidence and outcomes of ARDS in adult oncological patients in the ICU of a dedicated cancer hospital, as well as analyse the protective and risk factors associated with the mortality of this population.

Methods

Prospective cohort study. All patients admitted to the adult ICU from a dedicated cancer centre in southern Brazil from January 2012 to December 2013 were evaluated for ARDS. The ICU has eight beds and admits an almost exclusively oncologic population.

Inclusion criteria were: adult patients admitted to the ICU during the study period with cancer (solid or hematological) and who developed ARDS.

Exclusion criteria: Patients who were <18 years and those who stayed in the ICU for only <2 h (therefore, patients between 2 and 24 h were included) were excluded from the analysis.

Criteria and definitions:

- ARDS: Berlin Consensus Definition (15);
- Acute Kidney Injury (AKI): Any serum creatinine level higher than or equal to 1.5 times the baseline serum level, excluding patients with known prior renal disease (16);
- Sepsis: By the ACCP/SSCM Criteria (17), in use at the time of data collection;
- Vasoactive drug use: Any dose of norepinephrine, dopamine or vasopressin;
- Previous diseases (e.g. Chronic Obstructive Pulmonary Disease, Heart Failure, Chronic Kidney Disease): clinically defined by the healthcare team;

Clinical management (e.g. sedation, antibiotics, tracheostomy, glycemic control, vasoactive drugs, etc.), as well as the ventilatory strategy, were defined by the assistant ICU team (physician and respiratory therapist).

Descriptive statistical analysis was performed and percentages were expressed as frequency, mean and standard deviation. The analysis of baseline and epidemiological data and outcome were conducted using the Student's t-test, analysis of variance and Tukey's test, applying a significance level of $p<0.05$.

Multivariate analysis by logistic regression was performed to identify variables related to higher mortality.

The study was conducted in accordance with the recommendations in Resolution 466/2012 of the Brazilian National Council of Health. This study was approved by the Research Ethics Committee of the Universidade Estadual do Oeste do Paraná-UNIOESTE.

Results

During the study period, 729 adult oncological patients were admitted to the ICU. Of those, 489 required mechanical ventilation (MV). ARDS incidence (n=87) among cancer patients was 11.9% of the admissions and 17.8% in the subgroup that received MV.

Comparison between mechanically ventilated patients with and without ARDS showed that the first ones were more critically ill at ICU admission (higher APACHE II score), younger, had more hematological malignancies (mostly leukemias and lymphomas), higher rates of smoking and lower incidence of elective surgery as etiology. ICU length of stay, MV duration and mortality were significantly higher. Data on MV patients (ARDS or not) are shown in Table 1.

Among ARDS patients, 67.8% were admitted to the ICU due to medical causes; most common etiology was Pneumonia (57%), followed by extra-pulmonary sepsis (19%). Prevalence of previous radiation therapy or chemotherapy was 17% and 31%, respectively (even though only 4% had neutropenia). The most common administered antibiotics were Cefepime, Meropenem, Amikacin, Vancomycin and Teicoplanin. The most frequent complications during ICU stay were Acute Kidney Injury (AKI) (68.9%) and Ventilation-Associated Pneumonia (VAP) (64.4%).

Data analysis of the oncological patients that developed ARDS revealed that some factors were associated with higher mortality, including later-onset ARDS and a more positive fluid balance (supplemental archives [Table S-1], and Figures 2 and 3). However, logistic regression showed that only smoking and alcoholism were associated with higher mortality.

Table 1. Comparison of mechanically ventilated patients with and without ARDS

	MV, no ARDS	MV, ARDS	p-value
n	489	87	---
Male sex, %	65.5%	57.5%	0.189
Mean age (years) ± SD	60.9 ± 13.90	55.1 ± 17.18	< 0.001
Mean APACHE II at admission ± SD	24.4 ± 8.64	27.9 ± 8.94	< 0.001
Type of neoplasm, %	---	---	< 0.001
Solid	87.1%	65.5%	
Gastrointestinal (including liver)	41.1%	36.8%	
Urologycal	8.6%	8.0%	
Head and Neck (including thyroid)	17.5%	4.6%	
Oncohematologycal	12.9%	34.5%	
Lymphoma	3.1%	11.5%	
Leukemia	6.7%	17.2%	
Other previous comorbidities, %	---	---	0.357
None	34.4%	40.2%	
COPD	16.5%	10.3%	
Heart Failure	11.0%	10.3%	
Other neoplasia (in remission or not)	6.2%	4.6%	
Obesity (moderate or severe)	7.2%	5.7%	
Social habits, %	---	---	---
Tobacco smoking	27.8%	47.1%	< 0.001
Alcoholism	15.6%	17.2%	0.828
Cause of admission, %	---	---	< 0.001
Medical	46.2%	66.7%	
Elective Surgery	45.4%	23.0%	
Urgent Surgery	8.4%	10.3%	
Total MV duration, days, mean ± SD	3.4 ± 5.28	6.9 ± 6.41	< 0.001
ICU Length of stay, days, mean ± SD	5.9 ± 6.94	10.6 ± 10.16	< 0.001
ICU Mortality, %	55.3%	93.1%	< 0.001
Hospital Mortality, %	58.9%	96.6%	< 0.001

SD: Standard Deviation; MV: Mechanical Ventilation; ARDS: Acute Respiratory Distress Syndrome; ICU: Intensive Care Unit; COPD: Chronic Obstructive Pulmonary Disease; APACHE: Acute Physiology and Chronic Health Evaluation.

Supplemental File 1 - Table S-1. - Clinical and demographic data of patients with ARDS. n = 87

	Discharged alive (ICU)	Death ICU)	p-value
n	6	81	
Male sex, %	33.3%	59.2%	0.418
Age, years, mean ± SD	54.0 ± 18.67	55.3 ± 16.59	0.855
< 40	33.3%	14.8%	
41–60	50%	43.2%	
> 60	16.7%	42.0%	
APACHE II admission, mean ± SD	26.0 ± 7.13	28.0 ± 9.08	0.600
0–10	0	1.2%	
11–20	16.7%	28.4%	
21–25	33.3%	14.8%	
>25	50.0%	55.6%	
Type of neoplasm, %	---	---	---
Solid	66.7%	65.4%	0.701
Gastrointestinal (including liver)	50.0%	35.8%	0.797
Urologycal	0	8.6%	0.978
Oncohematologycal	33.3%	34.6%	0.701
Myeloma	0	3.7%	0.496
Lymphoma	0	12.3%	0.802
Leukemia	33.3%	16.0%	0.602
Recent chemotherapy, %	33.3%	32.1%	0.696
Recent radiation therapy, %	0	1.2%	0.860
Other previous comorbidities, %	---	---	---
None	50.0%)	39.5%	0.940
COPD	0	11.1%	0.867
Heart Failure	16.6%	9.2%	0.902
Hypertension	16.6%	33.3%	0.695
Other neoplasm (in remission or not)	16.6%	3.7%	0.656
Obesity (moderate or severe)	0	6.2%	0.778
Social habits, %	---	---	---
Tobacco smoking	50.0%	46.9%	0.781
Alcoholism	16.7%	17.3%	0.602
Cause of admission, %	---	---	---
Medical	66.7%	66.7%	0.654
Elective surgery	0	24.7%	0.377

(continued)

Supplemental File 1 - Table S-1. - Clinical and demographic data of patients with ARDS. n = 87

Urgent surgery	33.3%	8.6%	0.222
ARDS cause, %	- - -	- - -	- - -
Pulmonary	50.0%	80.3%	0.222
Pneumonia	50.0%	76.5%	
Broncoaspiration	0	1.2%	
Extra-pulmonary	50.0%	19.7%	0.223
Sepsis	50.0%	16.0%	
TRALI	0	1.2%	
Other	0	2.5%	
Time (days) from hospital admission to ARDS, mean ± SD	4.2 ± 10.57	9.0 ± 9.61	0.239
Time (days) from ICU admission to ARDS, mean ± SD	0.3 ± 0.51	2.7 ± 3.28	0.086
0	66.7%	34.6%	
1–2	33.3%	27.2%	
3–7	0	27.2%	
>7	0	11.0%	
Time (hours) of MV before ARDS, mean ± SD	0.2 ± 0.40	1.0 ± 1.60	0.227
0	83.3%	55.5%	0.368
1–24	16.7%	18.5%	0.663
25–72	0	21.0%	0.473
>72	0	5.0%	0.658
Fluid balance 1st day of ARDS, mean ± SD	1557.2 ± 1872.06	2956.1 ± 2481.61	0.181
< (−1.000)	16.7%	2.46%	
(−999) to (−300)	0	4.9%	
(−299) to (+300)	0	4.9%	
(+301) to (+1.200)	33.3%	12.3%	
> (+1.201)	50.0%	72.8%	
Units of PRBCs before ARDS, mean ± SD	1.7 ± 3.20	1.6 ± 2.99	0.937
0 (none)	66.6%	60.6%	
1–2	16.7%	19.7%	
>2	16.7%	19.7%	

MV: Mechanical Ventilation; ARDS: Acute Respiratory Distress Syndrome; ICU: Intensive Care Unit; COPD: Chronic Obstructive Pulmonary Disease; APACHE: Acute Physiology and Chronic Health Evaluation; PRBCs: Packed Red Blood Cells

Supplemental File 2 - Table S-2. Ventilatory parameters and outcomes of patients with ARDS. n = 87

	Discharged alive (ICU)	Death (ICU)	p-value
n	6	81	
Lowest PaO_2/FiO_2, mean ± SD	158.5 ± 96.41	147.9 ± 55.93	0.675
≤100	33.3%	20.5%	
101–200	50.0%	59.0%	
201–300	16.7%	20.5%	
Highest PEEP, cmH_2O, mean ± SD	14.2 ± 4.44	12.2 ± 4.06	0.272
≤5	0	0	
6–9	16.7%	28.4%	
10–14	33.3%	34.6%	
15–18	33.3%	23.4%	
>18	16.7%	13.6%	
Worst (lowest) Cstat, mean ± SD	33.0 ± 8.41	28.9 ± 8.33	0.116
<30	16.7%	53.8%	
31–50	83.3%	44.8%	
>50	0	1.4%	
Total MV duration, days, mean ± SD	8.2 ± 1.72	6.7 ± 6.63	0.224
1	0	1,2%	
2–5	16.7%	43.2%	
6–10	83.3%	19.8%	
>10	0	35.8%	
Vasoactive drugs, hours, %	- - -	- - -	- - -
0 (none)	0	2.5%	0.305
1–24	0	17.3%	
25–48	16.7%	18.5%	
>48	83.3%	58.0%	
ICU complications (others), %	- - -	- - -	- - -
AKI	16.7%	72.8%	0.016
Dialysis	0	22.2%	0.439
Pneumonia	33.3%	66.7%	0.229
Other infections	33.3%	18.5%	0.726
Lung biopsy	0	7.4%	0.885
ICU length of stay, days, mean ± SD	15.7 ± 7.65	10.2 ± 10.26	0.203
1	0	9.9%	
2–5	0	33.3%	
6–10	33.3%	19.8%	
>10	66.7%	37.0%	
Hospital Length of stay, days, mean ± SD	23.8 ± 7.30	19.4 ± 19.42	0.584

MV: Mechanical Ventilation; ARDS: Acute Respiratory Distress Syndrome; ICU: Intensive Care Unit; COPD: Chronic Obstructive Pulmonary Disease; AKI: Acute Kidney Injury; PEEP: Positive End Expiratory Pressure; Cstat: Static Compliance; APACHE: Acute Physiology and Chronic Health Evaluation.SD: Standard Deviation.

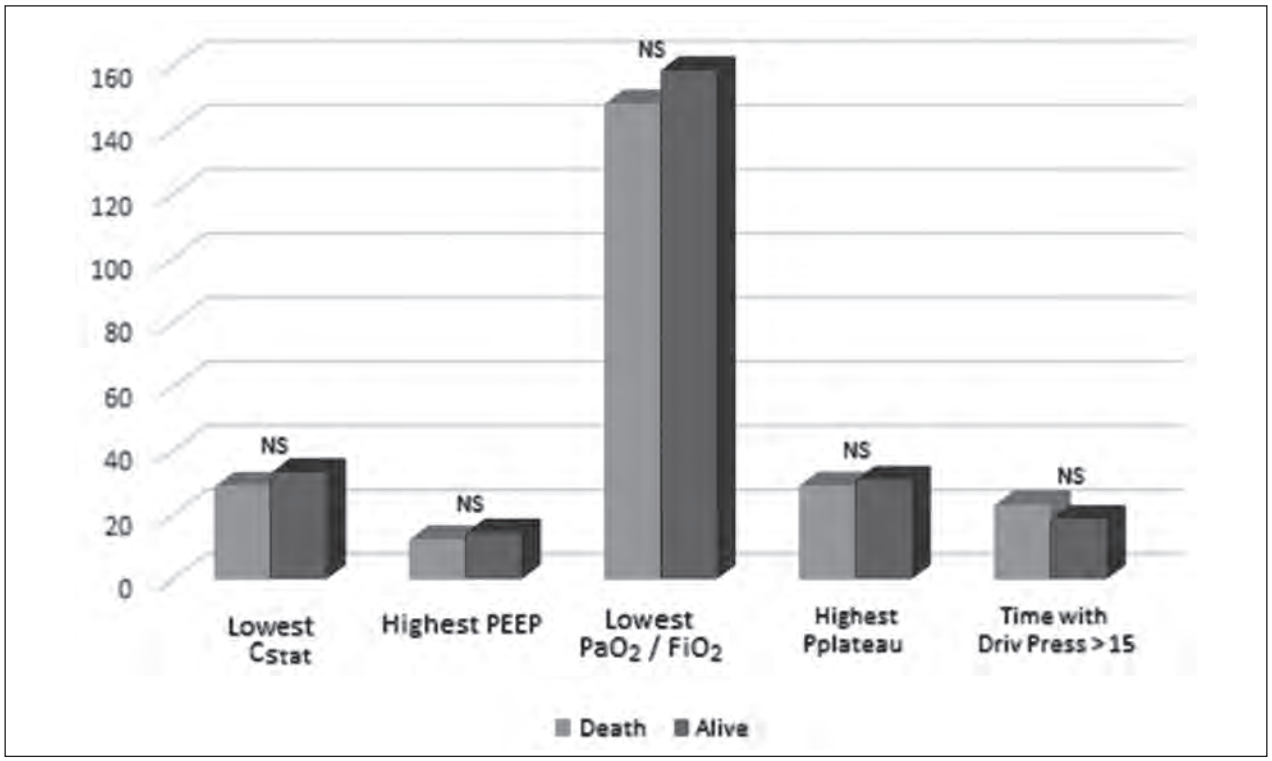

Figure 1. Ventilatory parameters of cancer patients with ARDS (n = 87)
Cstat = Static Complacency (in mL/cmH_2O); PEEP: Positive end-expiratory Pressure (in cmH_2O); PaO_2: Partial pressure of arterial oxygen; FiO_2: Fraction of inspired oxygen; Pplateau: Inspiratory plateau pressure (in cmH_2O); DrivPress: Driving Pressure (cmH_2O); NS: Non-significant.

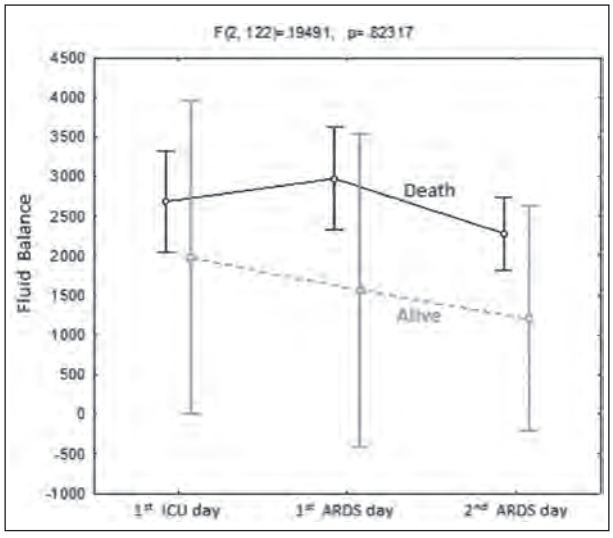

Figure 2. Fluid Balance in 1st ICU day, 1st ARDS day, 2nd ARDS day (n = 87)
Fluid Balance in mL/24h. ICU: Intensive Care Unit; ARDS: Acute Respiratory Distress Syndrome.

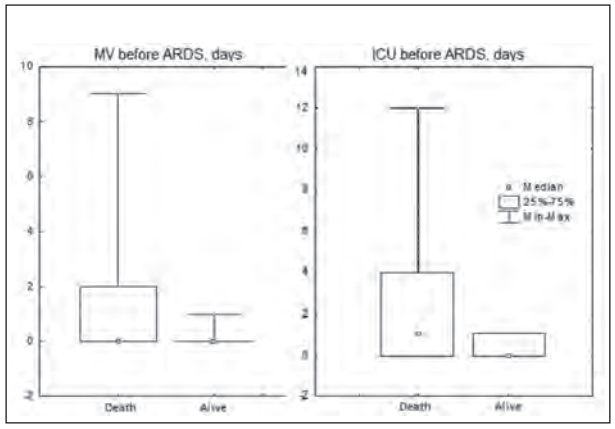

Figure 3. MV and ICU time (before ARDS) x Mortality (n = 87).
MV: Mechanical ventilation; ICU: Intensive Care Unit; ARDS: Acute Respiratory Distress Syndrome.

MV parameters were not significantly different when survivors and non-survivors were compared, although deceased patients had a higher incidence of pulmonary static compliance (Cstat) <30 mL/cmH_2O (supplement archives [Table S-2] and Figure 1).

Discussion

On this study, 11% of cancer patients admitted to the ICU developed ARDS, a similar percentage to that previously reported (11). The APACHE score was significantly higher in our ARDS patients than non-ARDS. Illness severity scores and acute physiologic alterations have been shown to predict mortality in ICU for both oncological and non-oncological patients (18-20). Besides, these scores are usually higher in oncological subjects (13).

Among ARDS patients, the prevalence of hematological cancer (mostly Leukemia and Lymphoma) was higher than non-ARDS, where the vast majority had solid tumours. Up to 11% of patients with hematological malignancies hospitalised will require ICU admission (21), and they are, in general, more severely ill, have higher rates of ARDS (22, 23) and mortality (24) than solid tumours patients. However, at least some of this result (higher mortality in onco-hematological patients) could be explained by the fact that most of the patients with solid tumours were admitted to the ICU for post-operative care after elective surgeries that have lower severity and risk of ARDS (25). When solid tumour and onco-hematological patients are compared, considering both being admitted to the ICU due to medical causes, such as acute respiratory failure or sepsis, ARDS incidence and mortality are similar (13).

ARDS patients' mortality in our study was exceptionally high, even when compared to other studies of oncological patients in the ICU (12). Although ARDS lethality went down recently, due to improving MV management and ICU care in general, the mortality remains high (26). Cancer-associated ARDS makes treatment more difficult due to poorly responsive infections related to immunosuppression, chemotherapy, radiation therapy and the involvement of lung tissue by neoplastic infiltrates (14, 15); for those reasons, mortality in cancer patients with ARDS is higher than non-cancer. We theorise that this particularly high mortality in our research could have been attributed to the fact that many patients that were included in this study ended up receiving exclusively palliative care, with therapeutical limitations, or other patients that rapidly died in a few hours (excluded from most similar studies). Besides, some studies of ARDS in oncological patients, even though retrospective or epidemiological, included mostly patients that were in randomised clinical trials (RCTs); consequently, they were highly selected patients, with usually strict inclusion criteria (12, 13). Thus, our study contributes and differentiates itself because we analysed 'real life' patients (without the effect of participation on RCTs). On the other hand, we should take into account the quality and intensity of care on ICU outcomes: sepsis mortality, e.g. has been shown to be higher in developing countries than in developed ones (27), and, at least in Brazil, it is higher in public hospital's ICUs than in private ones (28). Therefore, this work may provide thoughtful insight into the reality of ARDS in cancer patients, specially from developing countries, which may be different from optimistic results recently reported, that showed a reasonably similar mortality from ARDS in oncological and non-oncological patients (11, 12).

The main factors associated with higher disease severity and mortality in ARDS patients were the duration of MV and ICU stay prior to ARDS development (later onset of ARDS resulting in higher mortality), excessively positive fluid balance before ARDS development and the presence of clinical complications, particularly AKI. Patients with a positive fluid balance are more prone to pulmonary edema with worsening of pulmonary compliance interfering with gas exchange, unfavorable clinical outcomes (e.g. AKI) and higher mortality (29). However, the effects of positive fluid balance before or during ARDS are still controverse (30).

Duration of either ICU stay or time of MV before ARDS might point to different pathophysiologies and influence prognosis, including mortality and illness severity (31). Complications, such as nosocomial infections and AKI, have been described as factors of worse prognosis in ARDS, especially in oncological patients (12, 13, 32,33).

In our study, ventilatory parameters from ARDS patients that did not survive were usually worse than in the survivors: worse (lower) lung compliance, lower PaO_2/FiO_2 and higher driving pressure. On the other hand, PEEP was higher in the surviving patients (although lower mean PaO_2/FiO_2). MV strategies with lower tidal volume, plateau pressure, driving pressure and, possibly, higher PEEP have been shown to reduced mortality in ARDS in many different studies (26, 34-37), although 'very high' PEEP and alveolar recruitment strategies did not show any benefit (38). A previous study that analysed the impact of MV over mortality on oncological ARDS patients did not find prognostic association (13). Nevertheless, it is possible that because of the characteristics of that studied population (ARDSnet study subgroups), some variables might have been artificially different than those of daily practice (e.g. the median of the highest PEEP on this study was 8 cmH_2O, significantly inferior to our results and to those published by most epidemiological studies) (26, 39). Regardless of that, it has been found that in patients with hematological malignancies, those with lower PaO_2/FiO_2 and higher $PaCO_2$ had higher mortality rates (33). Lung compliance and gas exchange were found to be worse in oncological versus non-oncological ARDS patients, reflecting a possibly higher degree of lung involvement (14, 15, 19).

This study has several limitations (some of which are inherent to its nature), which may compromise the interpretation of the data. This was an observational study of a single centre (a specialised cancer hospital in Southern Brazil). This might not reflect the reality of most ICUs in our country or in the world, especially considering differences in outcomes regarding low/medium income versus high-income countries. In addition, the number of patients may not be large enough to answer questions about specific groups, such as the difference between solid cancer and onco-hematological patients. However, it was still comparable to many studies of the oncological patient in the ICU (24). Likewise, we did not have a control group of non-oncological patients developing ARDS. Due to being an observational study, the impact of evaluation and management strategies was not specifically studied, once the clinical decision was left to the medical and multi-professional team, according to local protocols and routines. However, the objective of the study was to evaluate the 'real life' situation of adult oncological patients who developed ARDS in the ICU of a dedicated cancer hospital in a developing country, and therefore, the design of the study was set up for this purpose.

Due to the study design, patients were only monitored until ICU discharge. For this reason, the late outcomes (including quality of life) were not evaluated in the present study.

Conclusions

In a population of oncological patients in a Brazilian ICU, the incidence of ARDS was high, particularly on medical and onco-hematological patients, with high mortality and complication rates. Patients with late-onset ARDS (after >24-48 h of ICU stay), more positive fluid balance on the 1st day of ARDS and lower lung compliance tended to have higher mortality rates.

Ethical approval and Consent to Participate

The study was conducted in accordance with the recommendations in Resolution 466/2012 of the Brazilian National Council of Health. This study was approved by the Research Ethics Committee of the Universidade Estadual do Oeste do Paraná-UNIOESTE. Accordingly, post-informed consent was waived, since this current study only describes the results of a population already previously treated.

Author's Contributions

PADD designed the study, analysed the data, wrote the manuscript; EMC, AT, KL collected the data, analysed the data, wrote the manuscript; RCS, TTC analysed the data, wrote and revised the manuscript. All the authors read and approved the final manuscript.

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A new frailty index as a risk predictor of morbidity and mortality: its application in a Surgery Unit

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Summary. *Background:* It's difficult to explain what *frail patient* means, because universal criteria for its identification and definition have never been drawn up. The whole scientific community is very interested to this issue of the potential effects that fragility may have on surgical and clinical outcomes. For this reason, we try to develop and validate the use of a new surgical frailty index (nsFI) to predict postoperative outcomes and mortality in General Surgery. *Methods:* The study was lead in the General Surgery Department of the "A.O.U. Mater Domini" of Catanzaro. The study was conducted using the database of the patients admitted in 2016. We calculated a score for each patient using data collected from medical records. Items of the Canadian Study of Health and Aging-frailty index (FI) were performed to develop a new frailty index to predict adverse postoperative clinical outcomes. Validation of our index was performed using the notorious mFI of Velanovich et coll., to confirm the proposed index. The resulting population was subdivided into 4 groups: not frail, mild, moderately and severely frail. Subgroups were created using gender, age, site of origin and type of pathology. Morbidity and mortality were evaluated after surgery. *Results:* A total of 481 patients were identified in accordance to inclusion criteria. According to our index 58% of this population was frail and 70% was over the age of 65. Biological frailty is correlated with the patient's origin area, so 61,7% came from rural regions. The percentage of frail men and women was the same. Malignant diseases were found in 71,01% of frail patients. 18,20% developed postoperative complications, while 1.32% died after surgery. This new surgical frailty index demonstrates good discrimination in our cohort (AUROC=0.74) better than previously modified frailty index (AUROC=0.54). *Conclusions:* This new surgical frailty index can be used to guide decision-making when applied on general surgery department. Furthermore, we have identified the identikit of surgical frail patients.

Key words: index, risk predictor, frailty, surgery

Introduction

Pre-operative risk evaluation is a fundamental tool to determine the patient's readiness for surgery, mortality and morbidity. Risk stratification for patients undergoing surgery is necessary for surgical planning, because this assessment permits to take a decision about whether to perform surgery or not, type and timing of the surgery. Moreover, it's useful to recognize patients who need a period of optimization before surgery. Furthermore is essential to forecast any complication and the needed procedures to prevent them (1).

Evaluation of frailty is an important variable for the estimation of perioperative risk in all patients (2-4). Nowadays frailty is considered as a well-characterized and validated method to objectively assess patient's fitness for surgery (5-6).

The World Health Organization in the last *World Report on Ageing and Health*, defines *frailty* as "extreme

vulnerability to endogenous and exogenous stressors that exposes an individual to a higher risk of negative health related outcomes" (7).

It is clear how frailty is consistently associated with adverse outcomes after surgery. The strongest evidences are in the association with increased 30 day, 90 day and 1 year mortality, post-operative complications and length of stay (8). This highlights the importance of early detection of frailty in the surgical diagnostic-therapeutic process. Score systems that are used to estimate the risk of surgery, are designed to predict mortality even if postoperative morbidity has been acknowledged as the major determinant of patient quality of life after surgery (1, 9-10). Traditionally, frailty has been measured by combining a patient's medical history, physical examination, and the assessment of physical and functional status (11-14). Many frailty definition tools were created for this purpose, but there is no one that has universal application. It is therefore necessary to tailor a specific tool for each medical area and especially for each surgical area.

One of the most famous tools in surgical research is that one created by Velanovich and colleagues that mapped the 70 variables included within the frailty index (FI) proposed by the Canadian Study of Health and Aging (CSHA) onto the American College of Surgeons National Surgical Quality Improvement Program (ACS-NSQIP) database to develop a modified frailty index (mFI) consisting of 11 preoperative variables measuring patient frailty (15).

The limit of this score system is the necessity to retrieve informations from American databases, but not all Countries have an available patient's database. For this reason, we have devised a score based on timely and rapidly detectable data from the patient's clinical record at the time of hospitalization. Moreover, Velanovich score doesn't incorporate surgical-specific informations to stratify patient's risk. For this reason it is not specific to any surgery in particular. In our opinion, this score put more attention on the general clinical problems of the patient, in despite of the surgical variables affecting the outcomes that obviously differ depending on the type of surgery the patient is facing.

Considering this background, it is necessary to develop a strong and clinically applicable preoperative frailty model that incorporates procedure-specific information to stratify patient's risk. (12, 16-20).

The aim of the current study was to develop and validate a clinically relevant frailty index using a dataset of patients undergoing general surgery. Additionally, we wanted to compare the predictive power of the proposed new surgical frailty index (nsFI) to existing frailty indices including the mFI.

Moreover our assessment will be performed on a young and old population, because there are several studies that investigate the effect of frailty on clinical and surgical outcomes, but most of them are limited to assessing the fragile elder. Most of the fragile population are over 65 years of age; nevertheless, it is also important to evaluate the effect of fragility on the younger population for the greatest impact they have on society.

Materials and Methods

The study was lead in the General Surgery Department of the "A.O.U. Mater Domini" of Catanzaro. The current analysis was performed using data drawn from clinical records of hospitalized patients from 1st January 2016 until 31st December in General Surgery Department. Among the informations extracted from clinical records, there were basic data such as age, sex, area of origin, type of disease, performed surgery, and post-operative complications. The main interventions considered for this study were surgery of the colon, breast, thyroid, kidney, stomach, pancreas, bile ducts, wall defects. Inclusion criteria were the department of origin and age over 18. For each patient was calculated a new surgical frailty index (nsFI) and modified frailty index (mFi) according to Velanovich. Using the list of 70 items from the Canadian Study of Health and Aging-frailty index (CSHA-FI), we selected the only ones that, according to the Author's experience, have the major impact on comorbidity and mortality after general surgery. We considered only factors that are able to increase the intraoperative risk, the complications and their severity, and the complexity of postoperative intervention and management. The items considered to be of greater value were crossed with data detectable by clinical records. An 11-element system was derived, as shown in Table 1. Each item had equal

Table 1. Items of the new surgical frailty score

Item		Variable	Score
1	Functional state	Independent	0
		Dependent	1
2	ASA class	1-2	0
		3-5	1
3	Presence of ascites	No	0
		Yes	1
4	Disseminated cancer	No	0
		Yes	1
5	Renal insufficiency or dialysis	No	0
		Yes	1
6	Stoma	No	0
		Yes	1
7	Urinary incontinence	No	0
		Yes	1
8	Difficulty in eating	No	0
		Yes	1
9	General mental health problems	No	0
		Yes	1
10	Anti- platelets Therapy	No	0
		Yes	1
11	Multiple drugs	No	0
		Yes	1

weight in the scoring index and it was considered as dichotomous variable, so for each variable could be attributed a score of 1 (yes) or 0 (no). The maximum expected score was 11. Patients were categorized into four groups based on their score: not frail (0 pt), mild (1 pt), moderate (2 pt), high frail (>3 pt).

For each patient was calculated a mFI according to Velanovich et al. The primary outcome of interest was the development of either a postoperative complication or postoperative mortality within 30 days of surgery. Postoperative morbidity was defined using a composite measure for postoperative complications that included surgical site infections, pneumonia, need for intubation, ventilator dependence, venous thromboembolism (pulmonary embolism or deep venous thromboembolism), acute renal failure, urinary tract infections, myocardial infarction, bleeding and sepsis.

Categorical data were reported as whole numbers and percentages and were compared using Pearson's chi-squared test.

Results of the comparison between the two methods were evaluated with area under the receiver operative characteristic curve (AUROC) statistics. Validation of the proposed index was performed using a leave one out cross-validation methodology. Statistical significance was defined by a p value of <0.01. All statistical analyses were performed using XLSTAT statistical software.

A score for linear trend in log odds (18) was used to assess the relationship between FI and postoperative mortality and morbidity.

Results

A total of 536 patient's records were identified in the database of hospitalized patients in 2016; 456 were eligible for the study. When a patient had assigned multiple folders due to different hospitalizations, only the first folder was considered and the others were used for calculating complications and mortality. The folders not included in the study belonged to patients who did not comply with inclusion criteria or did not received surgery. Moreover, we eliminated folders in which important data were missed. The median age of the study population was 62 years (IQR: 48-71) with a slight majority of female (n=254, 55.7%) (Table 2).

The most common site of origin was rural area (n=342, 75%), followed by urban area (n=114, 25%). There was quite the same amount of patients with malignancies (n=207, 45,39%) and benign pathologies (n=249 54,6%).

Validation of the nsFI was performed comparing it with mFI for the same group of patients. The nsFI demonstrated a good discrimination with a corresponding AUROC of 0.74 better than mFI, which demonstrated poor discrimination with a corresponding AUROC of 0.54 (p<0.001).

According to nsFi we found that 58,33% of the population of the study could be considered frail; in particular, the 22,59% was mild frail, the 17,32 % was moderate frail and 18,42% strong frail.

The most fragile patients are the older ones, in fact, the 83,2% of the population >65 years is fragile, compared to 48% of those between 50-65 years and

Table 2. Baseline patient and frailty characteristics evaluated with nsFI score

Characteristics	Not frail	Mild frail	Moderate frail	Severe frail
	41,67%	22,59%	17,32%	18,42%
Age				
0-50	80,65%	12,90%	3,23%	3,23%
50-65	52,00%	24,00%	12,00%	12,00%
>65	16,8%	19,57%	20,45%	43,18%
Sex				
Male	42,08%	19,80%	17,33%	20,79%
Female	41,34%	24,80%	17,32%	16,54%
Area				
Urban	51,75%	20,18%	13,16%	14,91%
Rural	38,30%	23,39%	18,71%	19,59%
Pathology				
Benign p.	52,21%	16,87%	15,26%	15,66%
Malignancies	28,99%	29,47%	19,81%	21,74%
Complicances	47,72%	23,06%	14,75%	14,48%
Mortality	33,33%	0,00%	16,67%	50,00%

of the 19,36% of patients under the age <50 (p<0.001; OD: 15).

Rural area is another determinant of frailty; in fact, 48,25% coming from urban area is frail, indeed 61,7% of rural area is frail (p<0.05, OD: 1.72); sex does not affect the determination of fragility as shown by OD: 0.96.

Particularly influential is the nature of the disease. Fragile patients suffer most from cancerous pathologies (71.01%; OD: 2.67).

Complications and mortality were compared among different grades of frailty. The increase of fragility degree was linked to an increase of postoperative complications and mortality.

Frailty is an important risk factor for complications and mortality; 85,54% of frail patients had complications after surgery while just the 14,46% of not frail patients had the same complications, in fact postoperative complications were developed in 20.48% of mild frailty, in 26.51% of moderate frailty and in 38,55% of strong frailty (p<0.001 OD: 5.96). Frail patients had major mortality, in fact 66.66% of death were frail, indeed 33,33% were not frail (p<0.05; OD: 1.45).

Discussion

Fragility is a physiological syndrome characterized by a reduced functional reserve and stress resistance, caused by a cumulative decline in several physiological systems, loss of homeostasis and consequent clinical instability and tendency to worse health manifestations (21).

There is a wide literature on the definition of the frail patient, that comes largely from the geriatric field, because fragility strongly associates with aging.

In the hospital path the detection of fragility is primarily finalized to help the clinician to identify frail patients and consequently stratify them for different levels of risk before surgery. In scientific literature, there are different types of frailty condition index. One of the best known and useful is the Velanovich one, a 11-point modified frailty index (mFI) that use data collected from the ACS-NSQIP to identify patients at risk for adverse postoperative clinical outcomes including postoperative complications, increasing LOS, and postoperative mortality (15). This accumulating deficits model based on patient's history, is a very useful and practical instrument to assess preoperative frailty, but it has several limitations. First of all, this score is applied to a national database; therefore, if clinicians don't have a database and if this is not specific and complete, it can't be used. The selected elements included in the Velanovich index cannot be considered the most important and impactful for general surgery. These are too generic, and could be incorrectly used for a fragile patient evaluation in a precise surgical specialty. Our goal, however, is to create a more specific index that is suitable for the type of surgical procedure conducted and in our case we are talking about surgery related to colon, breast, thyroid, kidney, stomach, pancreas, bile ducts and wall defects surgery.

Our nsFI score is made of robust, easy to use, 11 points index; for its creation We used the CHSA-FI because it easily identifies patient risk factors using just their clinical history. Some elements, such as ASA class, pharmacotherapy, and stoma are not extrapolated from the 70 items of CSHA-FI. These have been introduced into our index because, according to the authors, they have fundamental importance in fragility determination. The novelty of our study is also in the

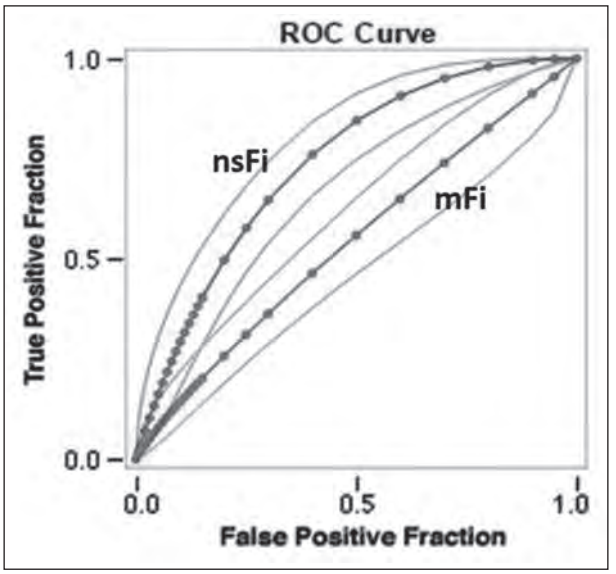

Figure 1. Roc Curve Comparison of the area under the curve of the nsFI (0.74) and the mFI (0.54)

index application, in fact all informations needed to define the score have been obtained from the patient's clinical records.

The study conducted demonstrates that our nsFI shows an improved discrimination and is more accurately able to risk-stratify patients undergoing general surgery when compared with the reference index (mFI). The new index of surgical fragility has demonstrated excellent ability to discriminate biological fragility. Comparing the two AUROCs (Figure 1), it can be seen that the nsFI is a better index than the Velanovich's mFi, which in itself showed little discriminatory ability.

Data suggests that the proposed nsFI is an accurate and easy-to-use risk stratification tool that can be used primarily from clinical folder analysis. Through a preoperative clinical evaluation, it is possible to predict the patient's risk of developing a post-operative adverse clinical outcome after surgery.

Conclusions

The study has shown that the fragile patient phenomenon is very common and important because about half of the patients hospitalized in a general surgery department are fragile. The typical identity of a fragile patient is elderly, coming from rural areas with cancer.

Our study also strengthens the data already found by Velanovich et al. on the fragility of youth; We calculated that a patient of five was frail. The implication here is that although fragility has been studied almost exclusively in older adults, it can be found even in younger adults. This younger and more fragile group has not received much attention in the literature. Further studies will have to be done to better investigate this aspect.

Several studies have analyzed the region impact on fragility but no one has ever focused on defining the effect on surgical outcome (22). Our data is in line with Italian rural realities where there is a smaller amount and less access to health services, and this is accompanied by a lesser awareness of the population living in these areas.

As for the type of pathology, it is easy to understand how malignancies are more complex because they alter the entire homeostasis of the patient. Not least is the effect that the same malignancies produce on the psychological sphere of the patient (23).

This study shows that the evaluation of fragility, based on a simple score determined by the patient's history, is associated with the occurrence of 30-year postoperative morbidity and mortality.

The effects of fragility seem to be more important in postoperative morbidity rather than mortality. The interpretation of all these studies is that fragility is a risk factor for complications and mortality after surgery.

Author contribution

Rosario Sacco, Antonietta Condoluci, Giuseppe Sammarco, Lucia Curto: Concept and design of study, data collection, data interpretation and analysis, drafting, revision, approval of final manuscript.

Michele Ammendola, Roberto Romano, Giuseppina Vescio: Study design, data collection, revision, approval of final manuscript.

Nikolaos Filiotis, Vincenzo Orsini: revision of final English form, critical revision of the entire text.

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Involvement of large rearrangements in *MSH6* and *PMS2* genes in Southern Italian patients with Lynch syndrome

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Summary. *Background and aim of the work:* The Lynch Syndrome (LS) is associated with germline mutations in one of the MisMatch Repair (MMR) genes. Most of germline mutations are point variants, followed by large rearrangements that account to 15-55% of all pathogenic mutations. Many study reporting the frequency of large rearrangements in the *MLH1* and *MSH2* genes were performed, while, little is known about the contribution of large rearrangements in other MMR genes, as *PMS2* and *MSH6*. Therefore, in this study we investigated the involvment of large rearrangements in *MSH6* and *PMS2* genes in a well-characterized series of 20 LS southern Italian patients. *Methods:* These large rearrangements are not usually detected by methods of mutation analysis, such as denaturing high-performance liquid chromatography (DHPLC) and direct DNA sequencing, but they are detectable by a known technique as the Multiplex Ligation-Probe Dependent Amplification (MLPA) assay. *Results:* No large rearrangements were identified in *MSH6* gene; instead, a large rearrangement was identified in *PMS2* gene. A large duplication including the exons 3 and 4 of the *PMS2* gene was identified in a patient who developed a rectum carcinoma at 45 years of age, an endometrial carcinoma and a vaginal cancer at the 65 years of age. *Conclusion:* We can affirm that the detection of large rearrangements in the *MSH6* and *PMS2* genes should be included in the routine testing for Lynch syndrome, especially considering the simplicity of the MLPA assay.

Key words: Lynch syndrome, HNPCC, *MSH6* gene, *PMS2* gene, *MMR* genes, large rearrangements, large duplication, genetic testing of Lynch syndrome

Introduction

The main hereditary gastrointestinal cancer syndromes (1) include the Familial Polyposis Adenomatous (2, 3), PTEN Hamartoma Tumor Syndrome (4), Peutz Jeghers (5) and Lynch Syndrome (6). Mutations in MisMatch Repair (MMR) genes are responsible for the early onset of colorectal cancer in Lynch syndrome (LS) (6). Germline mutations in *MLH1, MSH2* and *MSH6* genes account to 70-80% of LS cases, while a minor contribution (about 10-30%) is given by mutations in the *PMS2, MLH3* and *MSH3* genes (7-9). The mutations are distributed heterogeneously along each MMR gene, denoting the absence of "hot spots" mutations. Regarding to nature of germline mutations, most of these are point variants, followed by large rearrangements that account to 15-55% of all pathogenic mutations (10). Such alterations are mainly due to the presence of highly repeated sequences such as Alu sequences, which driver the recombination processes (11). A higher percentage of these rearrangements (deletions or duplications) are present in *MSH2* gene (20%) (12, 13); also in *MLH1, MSH6* and *PMS2* genes several large rearrangements were described in international literature (14). Molecular screening in suspected LS families attempted to find relationships

between a particular phenotype and a mutation in one of *MMR* gene (15). Although, the correlation genotype-phenotype for LS was not clarified to date (16), it is possible to affirm that the classic forms of LS, characterized by a early onset age of tumor (about 42 years) high penetrance and high degree of microsatellite instability (MSI) (17) were associated with point mutations in *MSH2* and *MLH1* genes. While, *MSH6* point mutations were reported in the literature as causing an "attenuated" forms of LS, with a later onset of tumor (18). Finally, point mutations in the *PMS2* gene were reported to cause early onset of tumors, that showed microsatellite instability but with different somatic features (19). Instead, the large rearrangements in any *MMR* genes (*MLH1, MSH2, MSH6* and *PMS2*) cause a similar clinical phenotype of disease, that is corresponding to classic forms of LS (20). These large rearrangements are not usually detected by methods of mutation analysis, such as denaturing high-performance liquid chromatography (DHPLC) and direct DNA sequencing, but they are detectable by a known technique as the Multiplex Ligation-Probe Dependent Amplification (MLPA) (12) assay. So far, many large rearrangements in the *MLH1* and *MSH2* genes were described as responsible of Lynch syndrome phenotype, while, little is known about the identification of large rearrangements in other *MMR* genes, as *PMS2* and *MSH6*. In this study we researched the large rearrangements in *MSH6* and *PMS2* genes in a well-characterized series of 20 LS southern Italian patients already negative for point mutations in the *MLH1, MSH2, MSH6, PMS2* and *MLH3* genes and for large rearrangements in the *MLH1* and *MSH2* genes. Identification of mutation responsible to LS phenotype, it is important in order to not exclude from the prevention and treatment program the subjects at risk of developing an early colon cancer.

Case report

In this study, the DNA of 20 selected subjects were analyzed by MLPA analysis to detection of large rearrangements in two *MMR* genes, *MSH6* and *PMS2* genes. These twenty subjects of Italian origin, 12 selected by the diagnostic criteria of Amsterdam (21) and 8 by the Bethesda guidelines (according to MSI high status) (22, 23) were recruited from several health centers in Southern Italy. Furthermore, as negative controls we collected 7 healthy samples from Clinical Department of Laboratory Medicine of our Hospital (Federico II of Naples). All patients received genetic counseling and gave their written informed consent to participate in this study. The detection of large genomic rearrangements in *MSH6* and *PMS2* in our selected patients was performed on genomic DNA using the SALSA MLPA P008-B1 PMS2 kit -Lot B1-0112 and P072-C1 MSH6 kit (MRC-Holland, Netherlands) according to the manufacturer's instructions. No large rearrangements were identified in MSH6 gene; instead, a large rearrangements was identified in *PMS2* gene. A large duplication including the exons 3 and 4 of the *PMS2* gene was identified in a subject (our number 1363) who developed a rectum carcinoma at 45 years of age, an endometrial carcinoma and a vaginal cancer at the 65 years of age. Figure 1A. For all patients, MLPA results were confirmed in three independent experiments. For the subject with our number 1363 and 7 negative references, we performed other two MLPA experiments using a 4 fold reduced amount of Ligase65 enzyme (0.25 µl/reaction), as suggested data sheet of P008-B1 PMS2 kit, Fig. 1B.

Discussions

Twenty subjects belonging to families with clinical diagnosis of LS were selected for this study. Of these twenty families, twelve meet the criteria of Amsterdam and eight showing an atypical phenotype were selected by MSI status on DNA extracted from tumoral tissue (data not shown). We performed the detection of large rearrangements in *MSH6* and *PMS2* genes in these LS families already negative for point mutations in *MLH1, MSH2, MSH6, PMS2* and *MLH3* genes and for large rearrangements in *MLH1* and *MSH2*. Therefore, in order to not exclude from the prevention and treatment program these subjects at risk of developing an early colon cancer we extended the research of mutation to analysis of large rearrangements in *MSH6* and *PMS2* genes that not are usually analyzed in genetic testing for LS. However, large rearrangements in these

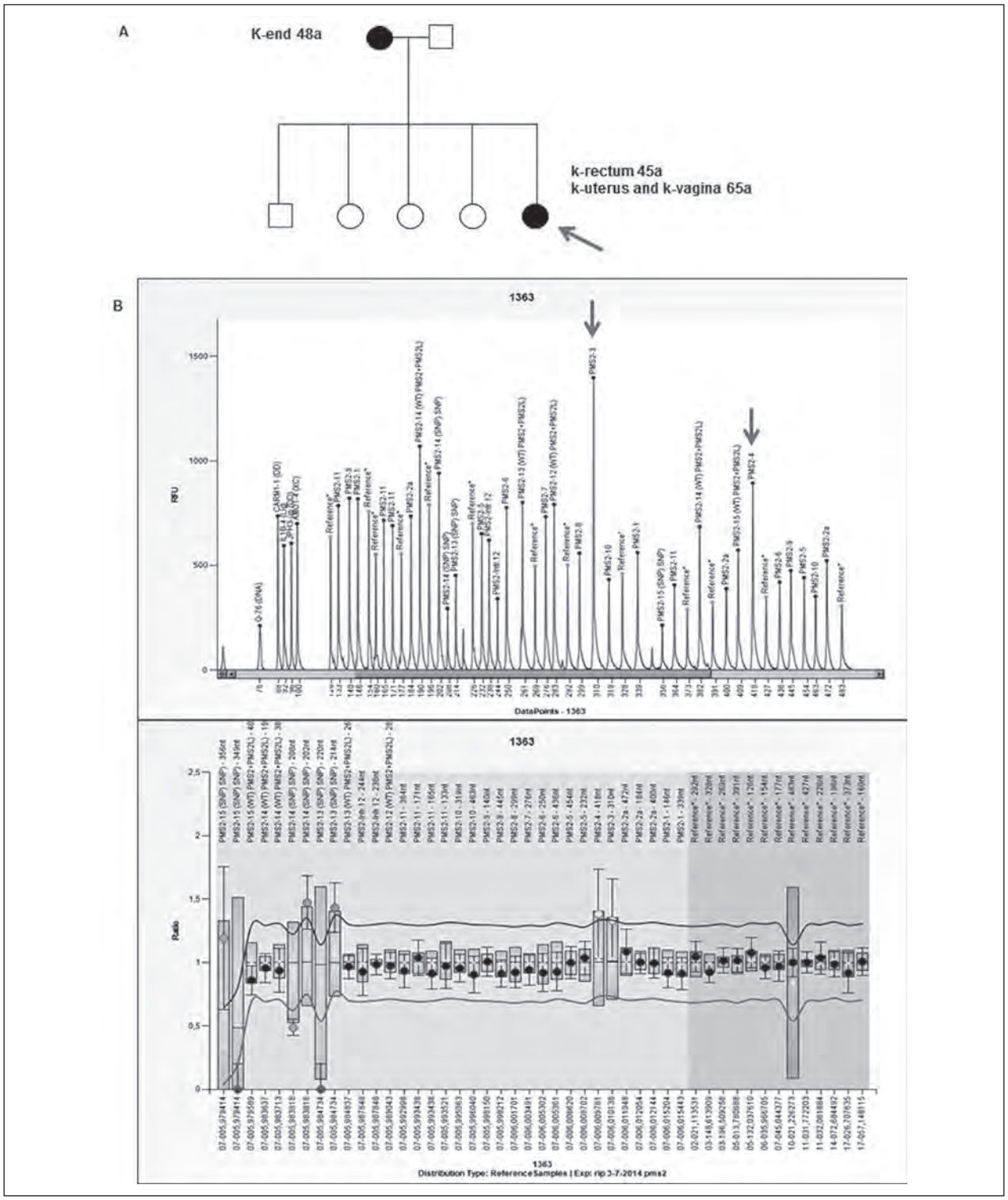

Figure 1. A. Family pedigree of our patient 1363 with a large duplication in PMS2 gene. Symbols and abbreviations used are denoted as fellow: arrows, index case; black symbol, colorectal cancer or tumors associate with LS; Co, colon cancer; End, endometrial cancer; Vag, vaginal cancer. Number next to diagnosis denote age at onset. and **B.** Electropherogram and graphical analysis showing the large duplication including 3 and 4 exons of *PMS2* gene.

genes were reported in literature (14, 24). In this study, no large rearrangements were identified in *MSH6* gene among our LS subjects. Instead, we identified a likely duplication including the exons 3 and 4 of *PMS2* gene. This duplication was identified in our LS patient (n. 1363) that developed a rectal carcinoma at the age of 45 and later a uterine and vaginal carcinoma at the age of 65, Fig. 1A. Literature data indicate that monoallelic mutations in *PMS2* gene are responsible of LS phenotype characterized by the presence of multiple tumors (25). The low penetrance could be to explain by redundant function of *PMS2* protein in the MMR complex. This could explain the absence of a significant family history for the subject 1363. Unfortunately, due to limited availability of subjects 1363 and to difficulty of analyzing the *PMS2* gene (26) we were not able to performed other experiments to confirm the MLPA result. However, as suggested data sheet of SALSA MLPA PMS2 kit P008-B1 to confirm the obtained result we repeated the MLPA experiment using a 4 fold reduced amount of Ligase65 enzyme, to exclude that this duplication of 3 and 4 exons of PMS2 could be an artifact of MLPA reaction (Fig. 1B). This condition could to occur due to difficulty of analyzing the PMS2 gene also by MLPA reaction for the presence of numerous pseudogenes (27). In conclusion, we believe that are needed further molecular analysis to confirm the duplication identified in *PMS2* gene. However, we can affirm that the detection of large rearrangements in the *MSH6* and *PMS2* genes should be included in the routine testing for Lynch syndrome, especially considering the simplicity of the MLPA assay. Finally, this study reaffirms the importance to identify pathogenic mutations in LS families to facilitate pre-symptomatic diagnosis and to improve therapeutic pathway in order to promote a personalized medicine (28).

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Metaplastic breast carcinoma with osseous remnant post standard treatment of invasive ductal carcinoma: case report and review of the literature

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Summary. Metaplastic breast carcinoma (MBC) is a rare subgroup of breast cancers that behave more aggressive in comparison with other breast cancer subtypes. Among them, the osseous variant is the rarest variant. Histologically, it consists of a metaplastic component beside main adenocarcinoma component. Consequently, this extra metaplastic part of MBC can justify more aggressive and chemoresistant behavior of metaplastic breast carcinoma. We present a case of a middle-aged female with metaplastic breast cancer that following standard chemotherapy of invasive ductal carcinoma, modified radical mastectomy with axillary lymph node dissection was performed. Surprisingly, related pathology report referred only to the mesenchymal component. The optimal treatment of MBC is not well-known yet, and the current approach is paralleled with other IDC subtypes. Therefore, studies about the MBC biologic markers can demonstrate new treatment approaches. This issue can be a milestone in the management of MBC, which targeting mesenchymal component in systemic therapy can improve clinical consequences.

Key words: metaplastic breast carcinoma, osseous differentiation, treatment, remnant

Background

Metaplastic breast carcinoma (MBC) is an infrequent and histologically diverse group of malignancies that make up less than 1 percent of all kinds of breast cancers (1). Invasive ductal carcinoma was detected as the most common type of all breast cancers, followed by invasive lobular and medullary carcinoma (2). But, the incidence of MBC (based on WHO 2012 report) has increased steadily since 2000 (3). The prevalence of breast cancer with osseous/cartilaginous metaplasia is very rare that estimated to occur in only 0.003-0.12 percent of breast cancer cases (4). It is called heterogeneous because of various kinds of histologies that may co-exist beside main histology of adenocarcinoma (e.g. squamous, spindle, chondroid and less commonly osseous variants) (5). MBC cases in comparison with patients diagnosed with invasive ductal carcinoma (IDC) have higher-grade and larger tumors with less hormone receptor (HR) positivity and also less inclusion of regional lymph nodes (6, 7). Generally, the prognosis and optimal treatment blueprint of MBC is not well-known. Treatment of MBC is largely analogous to other IDC subtypes, but growing evidence depict that MBC is a distinct entity of breast cancers (8). We report our experience with clinical status of a 41-year-old female diagnosed with metaplastic breast cancer that developed sarcomatous-only remnant after receiving treatment paralleled with IDC.

Case presentation

A 41-year-old woman with past medical history of metaplastic breast cancer referred to our department for

management of localized recurrence. Her initial clinical presentation was as follows; a painless lump located in the upper outer quadrant (UOQ) of left breast detected two months earlier. She had no history of trauma or nipple discharge and there was no known family history of breast cancer. On clinical examination, no dimpling, changes of skin color or nipple retraction detected. Through palpation a firm and mobile lump, measuring 3.0 cm × 3.0 cm, revealed. Mammogram demonstrated one well-circumscribed, dense and round mass in UOQ of the left breast, measuring 3.2 cm × 3.1 cm, but no micro-calcification detected. The mass corresponded to category 5 according to the BI-RADS Mammography Lexicon classification (8). Breast ultrasonography depicted an oval-shaped, complex echoic lesion measuring 3.0 cm × 2.8 cm with undetermined margins in UOQ of the left breast. But, no axillary lymphadenopathy detected. Accordingly, the lesion graded as BI-RADS 5 (8). Thereafter, the patient candidate for excisional biopsy. Pathology reported as follows:

"Sections of breast mass showed sheets of highly malignant medium to large cells with vesicular anisonuclei and eosinophilic cytoplasm with rare duct formation intermingled with the osteoid formation. This histologic picture is in favor of metaplastic carcinoma with the mesenchymal osseous formation." (Figure 1).

As is clear, the patient was a candidate for adjuvant treatment; but she had known as a candidate for close follow up by her primary physician. Three months later, based on physician physical examination another breast lump detected in her left breast, adjacent to previous mass. Accordingly, she was referred to our department to manage local recurrence of breast cancer.

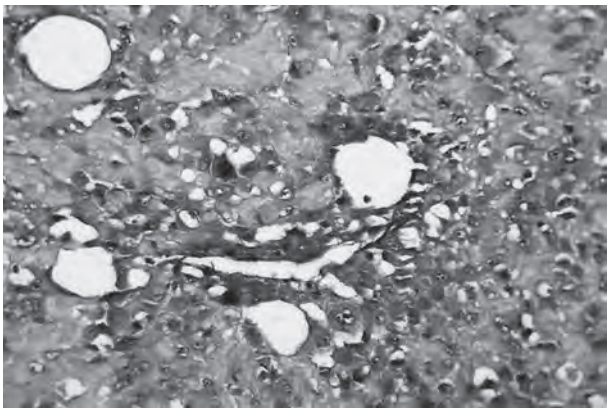

Figure 1.

On our clinical examination, no nipple retraction, skin dimpling or color change recognized. Palpation revealed a firm and immobile lump, measuring 5.0 cm × 4.0 cm in UOQ of the left breast. Furthermore, enlarged lymph nodes detected, measuring 2.0 cm × 2.0 cm, in her left axilla. The physical examination of her right breast and axilla was detected as normal. Mammogram depicted one poorly-defined, dense and irregular mass in UOQ of the left breast, measuring 7.0 cm × 5.2 cm, but no micro-calcification detected. The mass graded as category 5 according to the BI-RADS classification (8). Breast ultrasonography depicted one pear-shaped, complex echoic lesion measuring 7.5 cm × 6.0 cm with undetermined margins in UOQ of the left breast. An axillary lymphadenopathy detected with diffuse cortical thickening and loss of hilum. Accordingly, the lesion graded as BI-RADS 5 (8). Metastatic workup revealed no metastatic lesion. Consequently, the clinical stage assigned as IIIA (T3 N1 M0), according to AJCC 2010 reported TNM staging (9). Pathology review confirmed the initial diagnosis of metaplastic breast carcinoma. Immunohistochemistry (IHC) demonstrated that the cancer cells had a negative expression of P63, CK 5/6, ER, PR, c-erbB2 and the result of KI-67 reported as 20%.

Based on patient's demand for trying to save her breast, she was designated for neoadjuvant chemotherapy. Following chemotherapy with standard regimen of "Doxorubicin (60 mg/m², biweekly for 4 cycles) + Cyclophosphamide (600 mg/m², biweekly for 4 cycles) with Pegfilgrastim support, then Paclitaxel (80mg/m², weekly for 12 weeks)", the patient evaluated for breast conservation surgery, but because of small breast size, she was nominated for modified radical mastectomy (MRM). The specimen contained a firm white mass, measuring 8.0 cm × 6.5 cm × 5.0 cm, which showed bony consistency in some parts and the microscopic report was as follows:

"Numerous sections were taken from the tumor reveal a diffuse proliferation of polygonal cells with atypical nuclei with scattered bizarre cells, producing abundant osteoid and prominent woven bone" (Figure 2). Likewise, the tumor extended up to dermis but no lymphovascular invasion reported. Moreover, surgical

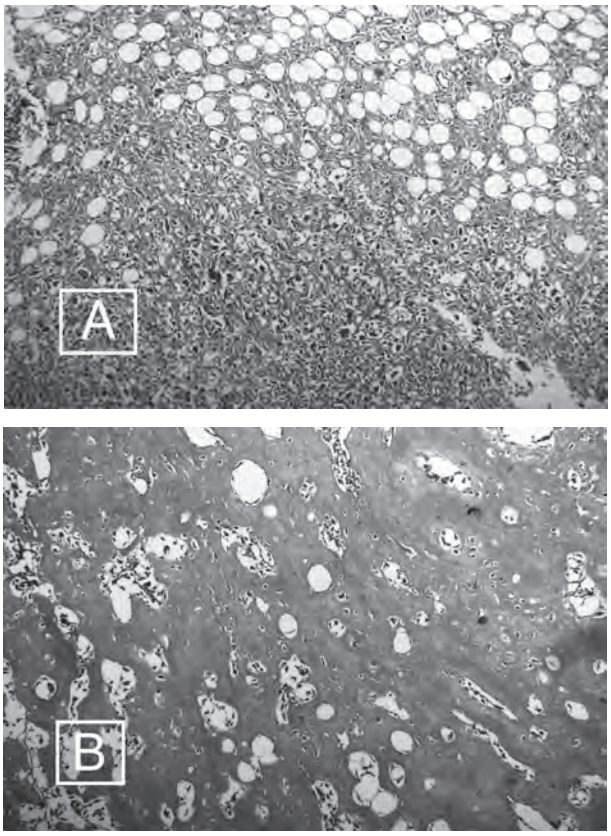

Figure 2.

margins were free and all ten left axillary lymph nodes dissected reported as reactive. The final diagnosis was consistent with osteosarcoma (no epithelial component was seen in the specimen) but granted that the patient had past medical history of MBC, the final diagnosis became "mesenchymal only MBC". The related IHC was as follows: negative result for P63, CK5/6, Bcl2, CD34, and B-catenin.

Thereafter, given that high probability of local recurrence, adjuvant chest wall, and regional nodal radiotherapy was done (5000 cGy in 25 fractions during 5 weeks). After 10 months, she is now in close follow-up setting, and fortunately, no evidence of recurrence was found.

Discussion

Metaplastic breast carcinoma was first characterized in 1973 by Huvos et al, as mammary ductal carcinoma combined with epithelial and sarcomatoid components (10). Nowadays, MBC constitutes 0.25-1.0 percent of all breast cancers (1, 11). MBC allude to a variety of histopathologies that contains both epithelial and mesenchymal components. Because it was not nominated as a distinct subtype until 2000, the current information about its characteristics is limited. The world health organization have categorized MBC into two distinct subtypes; 1) pure epithelial type, 2) mixed epithelial and mesenchymal type. The pure epithelial type subcategorized into adenosquamous carcinoma (ASC), squamous cell carcinoma (SCC) and adenosquamous with spindle cell differentiation (SPC); mixed epithelial and mesenchymal type subdivided into carcinoma with osseous and chondroid metaplasia (COC) and carcinosarcoma (CS) (12). MBC with osseous/cartilaginous components is one of the rarest subtypes of breast cancer that accounts for just 0.003-0.12 percent of all breast cancer subtypes (4). Among MBC cases with osseous/cartilaginous component, 51 percent of cases demonstrate cartilaginous metaplasia alone, 42 percent show both cartilaginous and osseous components, and the remainder 7 percent related to cases with osseous metaplasia alone (13).

The clinical presentation of MBC contains several properties that make it distinct from other IDC. The median age at diagnosis ranges from 48 to 59 years (14). Its growth rate is more than other IDC and generally represents larger than 2 cm at diagnosis. Despite larger tumor size, MBC involves regional lymph nodes less frequently than other IDC subtypes (15). In comparison with other IDC subtypes, lymph node involvement in patients with MBC does not essentially correlate with poor prognosis (16). Additionally, the expression of estrogen receptor (ER), progesterone receptor (PR) and c-erbB2 are lower in MBC in comparison with other IDC subtypes (17). The presence of metaplastic element beside epithelial element makes the prognosis of IDC poor, especially when it is prevailing component (18). Meanwhile, similar to soft tissue sarcoma, MBC demonstrates a high tendency for local recurrence and hematogenous spread to liver, lung, and bone (19).

Our patient represented many properties of MBC including large tumor size, lack of nodal involvement (at initial presentation), early loco-regional recurrence and triple-negative phenotype.

Breast cancer patients with MBC have a worse prognosis, in comparison with other IDC subtypes. Its 5-year survival ranges from 49 to 68 percent (20). Song et al. (21) compared prognosis of MBC subtypes and triple negative IDC (TN-IDC). The related result was as follows: the prognosis of TN-IDC was better than any subtype of MBC, with 5-year overall survival (OS) rate of 73.3% for TN-IDC in comparison with 50.0% in SCC, 56.3% for ASC, 40.0% in SPC and 75.0% in CS. Almost all MBC recurrences occur during initial five years, as long as recurrence curves of IDC steadily fall over time, suggesting that MBC recurrence may occur earlier than other subtypes of IDC (22). Some histopathologic factors that determine the poor prognosis of MBC including high cellularity, high mitotic activity, high nuclear grade and a high percentage of intervening spindle cells similar to sarcoma (23). Meanwhile, the presence of skin invasion, regional lymph node involvement with SCC as well as age less than 39 at presentation can be predictors of poorer outcome in patients with MBC (24).

The optimal treatment of MBC is not well-known yet, and the current approach is paralleled with other IDC subtypes. There are some uncharted issues regarding MBC that make its treatment results less efficacious than other subtypes of IDC. For instance, the pattern of MBC biologic markers are so different. I.e. they express HR and c-erbB2 less, whereas express EGFR-1 more than other IDC subtypes (25).Therefore, studies about the MBC biologic markers can demonstrate new treatment approaches. The second issue regards to various subtypes of MBC that seems to request distinct treatment approaches.

Growing evidence has appeared that demonstrate the distinct behavior of MBC. For instance, MBC tends to grow faster, involve regional lymph nodes less, spread hematogenous, and recur locally more in comparison with other IDC subtypes. This issues may be due to a metaplastic component of MBC. A report from the Mayo Clinic demonstrated the results of nine MBC cases that received standard IDC related chemotherapy regimens. The result was disappointing; just one partial response recorded (26). According to this, some modifications have been made in a few studies for the treatment of MBC with satisfactory results. For example, in a series reported by Hennessy et al, no recurrence recorded in three patients with MBC who had treated with Doxorubicin and Ifosfamide regimen (27). The second evidence relates to Gutman et al. (19) report that proposed sarcoma-directed therapy approach for MBC cases. Moreover, according to Brown-Glaberman et al. (28) report dramatic clinical response was seen with the sarcoma-based regimen in a patient with metastatic MBC status.

The result of our report can affirm the novel treatment approach. As mentioned our patient following receiving chemotherapy with the IDC-based regimen, revealed metaplastic only compartment in MRM related pathology report. It means that the epithelial component responded dramatically to conventional treatments of IDC, but the metaplastic component didn't. This issue suggests that changing attitudes regarding choices of systemic therapies can improve the results of MBC treatment.

Conclusion

Metaplastic carcinoma is a rare and heterogeneous subgroup of all breast cancers. These issues make its treatment approach uncharted. Current MBC treatment is paralleled with other subtypes of IDC, but there was some vague evidence in the literature regarding its behavior and type of recurrence that gave estimable clues to experts for running valuable studies to improve the treatment results. Consequently, targeting metaplastic component of MBC can improve the systemic therapy more efficacious in further clinical trials.

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A case of ependymoma with unusual radiological presentation

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Summary. Ependymomas are glial tumors, usually seen as intra-axial lesions in MRI. Here we report a case of an extra-axial lesion, resected as a meningioma; but pathology report was a Myxopapillary Ependymoma. Further evaluation detected another lesion in distal part of spinal canal too. So it seems logic that extra-axial tumors be managed with more caution.

Key words: ependymoma, meningioma, craniospinal irradiation, extra-axial, MRI

Introduction

Ependymomas are glial tumors that arise from ependymal cells within the CNS. Patients with Ependymoma are usually pediatric cases and the location of their tumors isintracranial, while in adultsEpendymomas are usually located inspinal canal (1).

Majority of the intracranial Ependymomas are located in the posterior fossa (infratentorial) usually arising from the floor of the fourth ventricle (1-3) (60%), while the remainder are located supratentorially(40%) (4). Supratentorial Ependymomas that are extra-axial are very rare, with only a few reported cases in the literature.

The World Health Organization (WHO) divides Ependymomas into 4 types on the basis of histologic appearance (5):

- WHO grade I: Myxopapillary Ependymoma, Subependymoma
- WHO grade II: Ependymoma (with cellular, papillary and clear cell variants)
- WHO grade III: Anaplastic Ependymoma

Case report

In September 2016, a 24 year old Iraqi girl came to our center becauseof nausea, imbalance, vertigo, and also nervousness which have been progressing since 2 years ago. A brain MRI without Gadolinium was done for the patient. A huge lobulated extra axial brain lesion, embedded on left Sylvain fissure with extension to Paraseller region was seen in this MRI (Figure 1).

Radiological diagnosis for this lesion was Meningioma.Due to the large size of tumor (5.8*3*3.8 cm), patient was referred for surgical resection in neurosurgery department. The removed creamy-brown rubbery specimen got examined under microscopic evaluation. Surprisingly, the primary pathologic report was suggestive for myxopapillary epandymoma. So, confirmatory IHC study by checking CK, GFAP, S-100vimentin and EMA was done, and the diagnosis was confirmed!

Theneurosurgeon referred the patient to Radiation-Oncology department for further evaluation and also complementary treatment. An MRI with and without Gadolinium for the whole spine of the patient was done. An abnormal signal 14*13 mm intradural extramedullary hetergenous enhanced mass lesion at the L4-L5 level was detected (Figure 2). The neurosurgeon refused to do a surgical resection for this new lesion. So we decided to do radiation treatment. In the first phase, a craniospinal irradiation (CSI) was done up to the dose of 36Gy in 20 fractions. A boost dose to the surgical bed of the resected lesion in the brain,

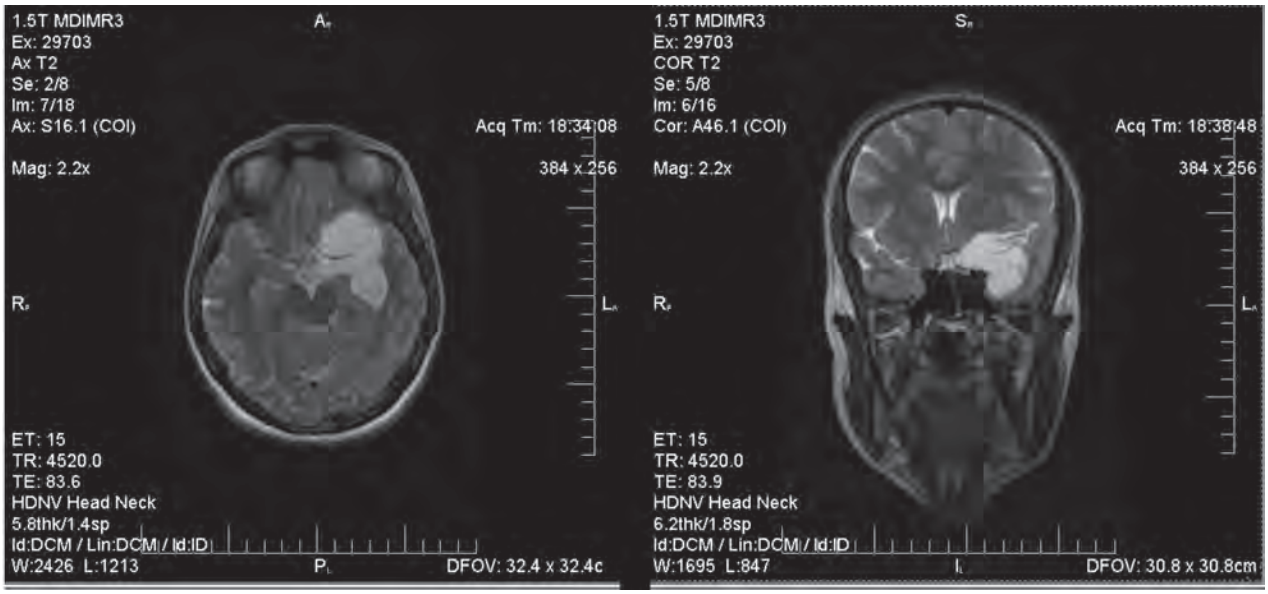

Figure 1. Pre-operative brain MRI (T2-FLAIR). A huge lobulated extra axial enhanced brain lesion, embedded on left Sylvain fissure with extension to Para seller region

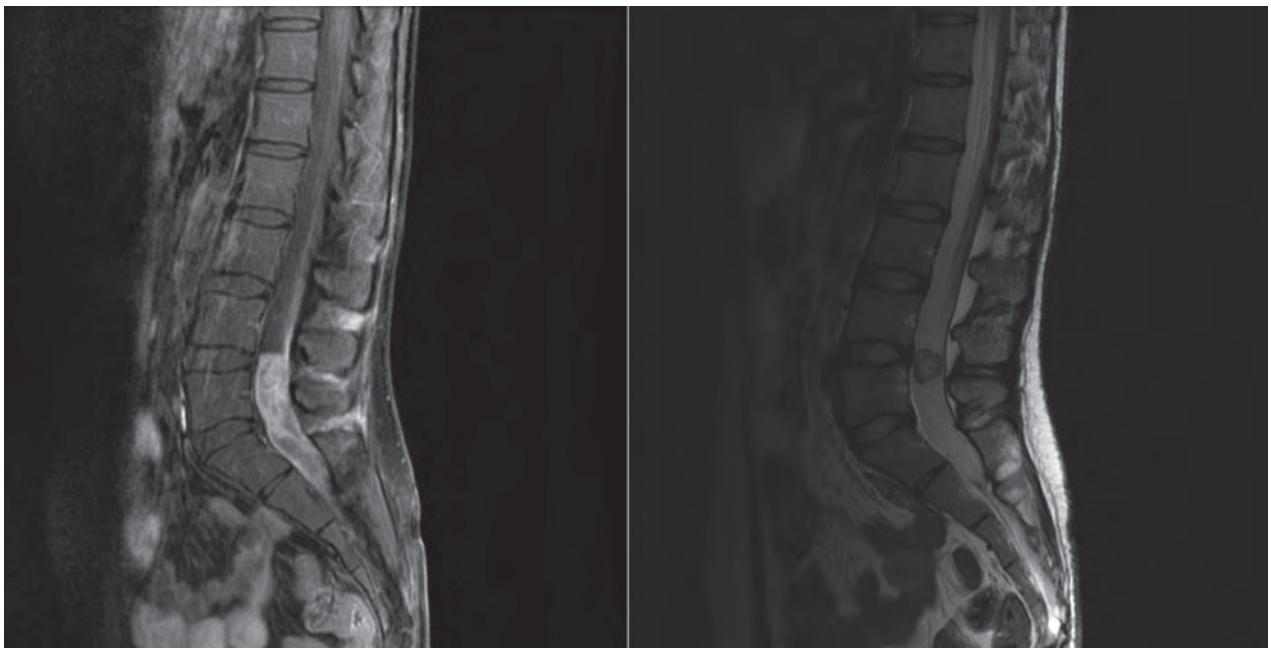

Figure 2. Intradural extramedullary hetergenous enhanced mass lesion at the L4-L5 level. (T1 with contrast [left] and T1 without contrast [right])

as well as to the lesion in the spinal canal was delivered up to the cumulative dose of 54Gy in 30 fractions. The patient tolerated the treatment very well. Control MRIs after treatment were done in Iraq. After about one year, the lesions are controlled without any progression.

Discussion

Ependymomas are usually intramedullary tumors. Extradural presentation with invasion of surrounding tissues is extremely rare (1).

Several hypotheses have been put forward to explain the origin of extra-axial Ependymomas with no connection to the ventricles. Fukui et al., proposed that such a tumor arises from glial rests in the subarachnoid space to produce an extra-axial mass (6). Hayashi et al., suggested that the tumor originates around the ventricle, grows and extends extramedullarly, followed by degeneration and necrosis of the ventricular portion of the tumor, leaving an extra-axial Ependymoma (7). According to Lyons et al., grossly nonvisible microscopic cellular tracts exist in development, between the ventricle and extra-axial Ependymoma that facilitate tumor extension into the subarachnoid space. These extensions subsequently regress (8). Vernet et al., postulated that tumors develop from intraparenchymal or subarachnoid ependymal cysts that result from disorders of migration from the germinal matrix (2, 6, 9). They represent primitive neuroectodermal tumors that have differentiated extensively along the ependymal lineage and might be the result of neoplastic growth within ectopic ependymal cells and are the consequence of a migration error (2, 10).

In conclusion, it should be taken into consideration that one of the differential diagnoses of the extra–axial lesions could be Epandymoma. Although there are no recommended guidelines for the management of extra-axial Ependymomas, it is advised that gross total resection should be done in such patients. Specially in children and young adults, it is more important when the patient is candidate for non-surgical treatment techniques like radiosurgery thereupon no pathologic evidence will be obtained. In these situations we should consider probable differential diagnoses like Epandymoma and if needed more evaluations like whole axis imaging should be performed (11).

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