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CLINICAL STUDY

Immunotherapy & radiotherapy – a future partnership
Focus on radiation induced lymphopenia (RIL) implications

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ABSTRACT
The hematological toxicity associated with radiotherapy is represented by neutropenia, anemia, thrombocytopenia, being associated with the increased risk of infection with opportunists, with fatigue and intolerance to effort, but also with the increased risk of bleeding. In the context of the preclinical and clinical results that mention the synergistic effect of the immunotherapy-radiotherapy association, radiation-induced lymphopenia (RIL) becomes an immunosuppression factor, a factor that would tip the fragile antitumor immunopotentiation-immunosuppression balance in favor of the immunosuppressive effect. Both the number of lymphocytes and the neutrophil/lymphocyte ratio (NLR) are prognostic and predictive biomarkers, providing information on the immune status of the host and on a possible response of the tumor to immunotherapy. Modern radiation techniques can increase the risk of lymphopenia by irradiating large volumes of tissue with low doses of radiation. In this context, a redefinition of the dose-volume constraints and the definition of bone marrow, lymphoid organs and lymph nodes not involved in tumors as organs at risk (OARs) is strictly necessary in the case of using irradiation through intensity-modulated radiation therapy (IMRT) techniques or volumetric modulated arc therapy (VMAT) for solid tumors that benefit from immune checkpoint inhibitor (ICI) therapy *(Ref. 22)*. Text in PDF *www.elis.sk*
KEY WORDS: lymphopenia, immunotherapy, radiotherapy, toxicity, spleen, nodes irradiation.

Introduction

Radiotherapy, a treatment historically considered as loco-regional with the potential to destroy tumors via mechanisms that mainly involve double-stranded DNA breaks and to sterilizing possible metastatic dissemination from the lymph nodes draining the tumor, receives in immune checkpoint inhibitors (ICI) era a new attribution: to augment the effect of immunotherapy treatment in order to maximize the benefit of synergistic radiotherapy-immunotherapy associations. If until now limiting the doses received by healthy tissues was a priority with the exclusive aim of reducing the treatment related toxicities, the new concept must also take into account the immunosuppressive effect associated with irradiation, an effect that could reduce the benefits of the multimodal oncological treatment. The hematological toxicity associated with the depletion of progenitors, but also with chronic effects on lymphoid organs by limiting the vascular network and fibrosis can lead to neutropenia, anemia and thrombocytopenia. As a consequence, these toxicities can be associated with an increased risk of infection with opportunists, of bleeding or of asthenia. Modern irradiation techniques with modulated intensity offer a better conformation of the target volumes, but also are notorious for the "bath of low radiation doses" scattered in large volumes of healthy tissue. The limitation of bone marrow, lymphoid organs or of not involves lymph nodes irradiation should be considered a priority associated with management of solid tumors that currently benefit from immunotherapy. Such a strategy can tilt the frail balance in favor of the benefits (tumor destruction and immune potentiation) and limit immunosuppression via radiation-induced lymphopenia (RIL). It is necessary to refine the dose-volume constraints as a strategy to potentiate the synergistic effect of immunotherapy-radiotherapy (1, 2).

Radiation induced lymphopenia (RIL) – an underestimated toxicity

RIL is a subject of interest in relation to small doses of radiation delivered in large volumes of tissue, knowing that lymphocytes are the most radiosensitive cells in relation to the erythroid or myeloid lineage. Ceasire and collaborators mention the detrimental effect in survival of lymphopenia, considering that it can be a persistent and severe toxicity, affecting lymphocyte subpopulations differently, in relation to the mode of irradiation. The authors consider that the effect is different depending on the of lymphoid irradiated structure type. They also warn about the need of toxicity risk reevaluation (1, 3).

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The prognostic impact of RIL on overall survival (OS) was analyzed for patients with solid tumors in a systematic review, evaluating 20 studies included in the PubMed/MEDLINE databases. Grade ≥ 3 lymphopenia induced by irradiation was significantly correlated with a reduction in OS, in brain, lung and pancreas tumors, the association being more obvious. The authors note the adverse effect of RIL on the prognosis, recommending the re-evaluation of lymphopenia reduction methods. It is considered that a radiation dose of 2 Gy is associated with a reduction in half of the survival fraction of lymphocytes. A dose only 1 Gy higher than the previously mentioned dose has the effect of a 90 % reduction in the survival fraction of lymphocytes. According to Nakamura and colleagues, the results are valid for both CD4+ helper T lymphocytes and CD8+ cytotoxic T lymphocytes. The same concept of the need to reduce the irradiation of circulating lymphocytes is debated by Yovino et al. Analyzing the radiation dose received by circulating lymphocytes during the radiotherapy of brain tumors, the authors report a mean dose to circulating cells (DCC) of 2.2 Gy, without significant differences between the conformal or intensity-modulated irradiation techniques. Regulatory T lymphocytes (T-regs) are still considered by some authors to be more radio-resistant. The asymmetric distribution in the organs as well as the different radio-sensitivity can explain the long-term imbalance on the immune system produced in the survivors of A-bomb. Attempts to manipulate the immune system to improve the therapeutic ratio based on the imbalance of immune-potentiation / immune-suppression is an old concept. Super agonist antibody TGN1412, tested in a phase I study in 2006 led to the death of 5 subjects, the clinical implementation of this agent being reported by Attarwala as "disaster" (4–10).

Irradiation has the effect of reducing naïve T cells with direct consequences in the decrease of the absolute number of lymphocytes and an increase in the proportion of T-regs, known to have the potential to create an immunosuppressive tumor microenvironment, but radiotherapy does not seem to affect the functioning of CD8+ lymphocytes. Splenic irradiation in abdominal tumors is also associated with an increased risk of lymphopenia (3, 11).

Lymphopenia in cancer – implication for immunotherapy

Solid tumors develop a number of methods to escape the host's immune response. Based on this concept, immune checkpoint inhibitor (ICI) therapy has brought substantial OS benefits in the last decade, melanoma and lung cancer being among the biggest winners of these innovative therapies. Inhibitors of cytotoxic T lymphocyte-associated protein 4 (CTLA-4), programmed death receptor 1 (PD-1) or its ligand (PD-L1) are agents that are already part of the routine clinical practice of oncology. These therapies favor the interactions between T lymphocytes, macrophages and tumor cells, potentiating the death of the malignant cell by reactivating the effect of T lymphocytes, thus through an indirect mechanism. The response rate of 20–40 % makes it necessary to identify biomarkers that prevent the administration of immunotherapy to non-responders or, if possible, to identify a strategy to convert immunologically "cold" tumors into "hot" tumors that will ultimately benefit immunotherapy identically to that of native tumors responsive to ICI. Lymphopenia and the neutrophil/lymphocyte ratio (NLR) have been proposed as a surrogate marker for the initial response to ICI.

The combined positive score (CPS) quantifies both PD-L1 expression on tumor cells and immune cells. This score, tumor infiltrating lymphocytes (TILs) count rate < 30 %, PD-L1 expression were associated with high NLR values in laryngeal cancer. Values of TILs > 30 % and CPS ≥ 1 were identified as being associated in this study with increased disease free survival (DFS). In the case of palliative radiotherapy, the data obtained from retrospective studies demonstrate a correlation of RIL with the negative prognosis for patients treated with ICI. Irradiation of the spine, mediastinum and chest wall was associated with a higher risk of RIL than radiotherapy of the brain, abdomen and pelvis. Absolute lymphocyte count (ALC) < 500 was associated with irradiation of the mediastinum and chest and with a shoulder greater than 5 fractions of irradiation. In the case of the association of ICI, a history of radiotherapy less than 3 months before the administration of immunotherapy was associated with an unfavorable prognosis (12–14).

Lymphopenia - implications of dose/volume parameters in radiotherapy

The subject of lymphopenia associated with irradiation returns today with the resumption of an old concept of over 100 years, that of using radiotherapy to limit the inflammation generated by pneumonia and more specifically the cytokine storm associated with the COVID-19 infection. A dose with anti-inflammatory potential of 0.3–1Gy delivered to the entire volume of the lungs can be associated with an increased risk of prolonged lymphopenia, especially in cases with lower pre-treatment lymphocyte count. Thus, limiting the volume of the irradiated lung and applying the concept of partial irradiation aims to limit the hematological toxic effects. Precautions are recommended regarding the definition of clinical target volumes (CTV) for radiotherapy during the COVID-19 pandemic, but these strategies can also be extrapolated in the general context of reducing immunosuppression through lymphopenia. In the case of unclear clinical benefits of elective lymph node irradiation, the use of an extended CTV volume should be reevaluated, as long-term lymphopenia is associated with possible severe consequences. For this reason, during the COVID-19 pandemic, it is recommended to avoid prophylactic radiotherapy of the pelvic nodes in localized prostate and bladder cancer, but also to approach prophylactic radiotherapy of the para-aortic lymph nodes in locally advanced cervical cancer with caution. Precautions are also recommended in the inclusion of the axillary lymph node in the irradiation field in patients with early breast cancer treated by wide excision or with negative sentinel nodes. A controversial topic is also the irradiation of internal mammary nodes for T3 and/or N1 cases, considered intermediate risk disease, but also in cases of central or medial breast tumors (15).

Also patient related factors such as advanced age or smoking, but especially baseline lymphocyte count are considered to be associated with an increased risk of RIL. For cases of breast cancer

treated with adjuvant radiotherapy, the mean dose received by the heart, the mean bilateral lungs doses, chemotherapy, surgery, but also the dose received by the whole body and the number of irradiation fields were factors associated with RIL (16).

A study including 711 patients receiving definitive RT for non-small cell lung carcinoma (NSCLC) identified larger gross tumor volumes (GTV) as a predictor of lymphopenia. Also, the administration of chemotherapy, stage III of the disease and the administration of two fractions of irradiation per day were factors correlated with the decrease in the number of lymphocytes. XRCC1 rs25487 polymorphism, a factor associated with the risk of lung cancer, is also identified as a predictor of RIL along with age and the volume of the lung receiving at least 5Gy (V5). Lower doses (long V1–V15) were, along with large GTV and lower lymphocyte nadir, predictive factors of reduced survival in esophageal cancer cases treated with neo-adjuvant radio-chemotherapy using the Dutch CROSS regimen (17–20).

It can be considered that low-dose irradiation of structures that contain large amounts of blood or that present high blood velocities may be associated with RIL. In this context, a justified fear is associated with the intensity-modulated radiation therapy (IMRT) and volumetric modulated arc therapy (VMAT) techniques, famous for spreading lower doses in large volumes of healthy tissue. If until now the risk of second cancer was the main reason to limit low dose bath, in the context of ICI therapies, the lymphopenia associated with the treatment benefits from increased attention, justifying the reduction of low doses received by large volumes. The spleen, an organ not routinely defined as OAR, is mentioned in the International Society of Pediatric Oncology (SIOP)-Europe Radiation Oncology Working Group report, as OAR in pediatric radiotherapy, being recommended to limit the mean dose to < 10 Gy and administer prophylactic antibiotic therapy in the case in which this dose is exceeded. Recently, a group of radiation oncologists from Bucharest University of Medicine published a retrospective study that associates V15 and maximum radiation doses received by the spleen with lymphopenia induced by chemo-radiotherapy. An increase in the mean dose received by the spine by 1 Gy is associated with a 1 % decrease in absolute lymphocyte count at nadir. The authors consider the spleen as a structure that must be defined as an OAR, as it is necessary to implement dose-volume constraints for this organ (21–22).

Conclusions

In the context of the association in clinical practice of immunotherapy and radiotherapy, in order to potentiate the synergistic effect and limit the immunosuppression associated with irradiation, it is necessary to implement some strategies to reduce RIL. Thus, the careful assessment of the need for elective lymph node irradiation, the imposition of dose-volume constraints to reduce the "low dose bath" associated with IMRT and VMAT irradiation and the definition of large vessels and the spleen as OARs are necessary to limit RIL and to contribute to the transformation of the immunotherapy-radiotherapy association into a success story.

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