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Mini review article

ASSOCIATION BETWEEN SERUM CYTOKINE LEVELS AND THE DEVELOPMENT OF ACUTE RADIOTOXICITY IN PROSTATE CANCER PATIENTS

VEZA IZMEĐU NIVOA CITOKINA U SERUMU I RAZVOJA AKUTNE RADIJACIONE TOKSIČNOSTI KOD PACIJENATA SA KARCINOMOM PROSTATE

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Abstract

Radiotherapy plays a significant role in the multidisciplinary approach to treating prostate cancer patients.

However, some of these patients may develop severe adverse effects after receiving radiotherapy that negatively affect their quality of life.

Radiotoxicity may manifest in the lower gastrointestinal (GI) tract by damaging the rectum or bowel, or genitourinary (GU) tract, causing symptoms due to urethral, bladder or prostate damage.

The probability of complications in normal tissue increases as the delivered radiation dose increases. However, there are patients with satisfactory dosimetric parameters who develop radiation toxicity and vice versa.

Prediction models that take into account additional parameters to identify patients most susceptible to developing toxicity may serve as essential factors toward personalized radiotherapy. The main objectives are morbidity reduction and life-quality improvement.

Changes in the cytokine levels could also be connected with the occurrence of acute gastrointestinal and genitourinary toxicity. Literature data indicate the association of numerous cytokines with the appearance of GI and GU toxicity. There is proof that TGF-β1 stimulates fibroblasts to generate extracellular matrix. According to the literature, IL-6 is regarded as one of the most important immune markers for predicting the radiotherapy-induced toxicity of normal tissues. Increased IL-6 concentrations in the serum during radiotherapy are significantly linked to a higher degree of acute genitourinary toxicity.

The goal of this work is to summarize the results of contemporary research in which the connection between the occurrence of acute radiation toxicity and changes in the cytokines levels in the serum during radiotherapy were examined, considering the great future potential of the use of toxicity prediction factors in clinical practice.

It can be concluded that radiation therapy, the development of an inflammatory process, and the occurrence of radiation toxicity are all related. However, further research with the aim of adequate stratification of patients for the development of an individualized approach to radiotherapy is required.

Keywords:
radiotherapy,
prostate cancer,
inflammation,
radiation toxicity,
cytokines

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Sažetak

U multidisciplinarnom pristupu u lečenju raka prostate radioterapija igra važnu ulogu.

Neki pacijenti sa ovom dijagnozom koji su lečeni radioterapijom mogu, međutim, razviti ozbiljne neželjene efekte koji utiču na njihov kvalitet života.

Akutna i kasna toksičnost se može manifestovati u donjem gastrointestinalnom (GI) traktu, razvijajući toksičnost creva i rektuma i genitourinarnom (GU) traktu, izazivajući simptome kao posledicu oštećenja uretre, mokraćne bešike i prostate.

Eskalacija doze zračenja takođe povećava rizik od komplikacija na okolnom zdravom tkivu. Međutim, postoje pacijenti sa zadovoljavajućim dozimetrijskim parametrima koji razvijaju toksičnost zračenja i obrnuto.

Predikcioni modeli koji uzimaju u obzir dodatne parametre za identifikaciju pacijenata koji će verovatno razviti toksičnost mogu biti ključni korak ka personalizovanom pristupu u radioterapiji sa modifikacijom doze zračenja, broja frakcija, kao i tehnika zračenja. Primarni cilj je smanjenje morbiditeta i poboljšanje kvaliteta života.

Pokazalo se da su izmene u nivou inflamatornih biomarkera u serumu, kao što su citokini, u pozitivnoj korelaciji sa akutnom gastrointestinalnom i genitourinarnom toksičnošću. Podaci iz literature ukazuju na povezanost brojnih citokina sa pojavom GI i GU toksičnosti. Postoje dokazi da transformišući faktor rasta beta 1 (TGF-β1) služi kao ključni medijator fibroze tako što aktivira fibroblaste za proizvodnju ekstracelularnog matriksa. Prema literaturi, interleukin 6 (IL-6) se smatra jednim od najvažnijih imunoloških markera za predviđanje radioterapijom indukovane toksičnosti normalnih tkiva. Povećane koncentracije IL-6 u serumu tokom radioterapije značajno su povezane sa višim stepenom akutne genitourinarne toksičnosti.

Cilj našeg rada je sumiranje istraživanja, sa akcentom na savremene reference, u kojima je ispitivana povezanost nastanka akutne radijacione toksičnosti i izmene nivoa citokina u serumu tokom sprovođenja radioterapije, imajući u vidu veliki budući potencijal primene faktora predikcije toksičnosti u kliničkoj praksi.

Može se zaključiti da postoji povezanost između radioterapije, razvoja inflamatornog odgovora i pojave toksičnosti zračenja. Međutim, neophodna su dalja istraživanja u cilju adekvatne stratifikacije pacijenata za razvoj individualizovanog pristupa u radioterapiji.

Ključne reči:
karcinom prostate,
radioterapija,
inflamacija,
radijaciona toksičnost,
citokini

Introduction

Prostate cancer remains a global public health concern worldwide, particularly in countries where the life expectancy is long enough to clinically manifest the disease (1).

The Globocan 2020 report says that prostate cancer is the second-most prevalent cancer and the fifth most common cancer-related death in men worldwide (2).

The newest statistical report from the Serbian Cancer Registry of the Institute of Public Health of Serbia "Dr Milan Jovanovich Batut" shows that prostate cancer was the third most common cancer type in men and the third most prevalent cancer-related mortality in Serbia in 2019 (3).

The prostate cancer mortality rate has significantly decreased with the invention of prostate-specific antigen (PSA) for screening. Along with the impressive number of its benefits, estimates suggest that up to 23 - 56% of men may undergo unnecessary diagnostic testing (over-diagnosis) or therapeutic interventions (over-treatment), for a disease that is highly unlikely to manifest clinically during their natural lifespan (1). Therefore, identifying patients who are most likely to contract the disease and benefit from early diagnosis and treatment is implicit in any screening effort. It prevents the deterioration of a patient's quality of life caused by unnecessary treatment.

The survival rate for prostate cancer has significantly increased due to effective treatments such as radiotherapy (RT), surgery, and hormonal therapy.

Radiotherapy in prostate cancer

In the multidisciplinary approach to treating prostate cancer, radiotherapy plays a significant role. Approximately two-thirds of prostate cancer patients should be treated with radiotherapy as initial treatment or later due to recurrence or disease progression (4).

Although the main goal of radiotherapy is to apply the appropriate dose to the tumor, surrounding healthy tissues receive some dose as well, with the consequent development of radiotoxicity.

Advanced radiotherapy techniques, such as Three-dimensional conformal radiotherapy (3DCRT), enable better preservation of normal tissue in comparison to previous techniques (5). Intensity-modulated radiotherapy (IMRT) and volumetric modulated arc therapy (VMAT) were implemented to further improve the dose distribution with the opportunity of escalation dose in prostate cancer patients up to 76 - 78 Gy and even more (**figures 1 and**

2) (6, 7). For patients receiving radiotherapy, image-guided radiotherapy (IGRT) provides a more precise method for dose distribution (8, 9).

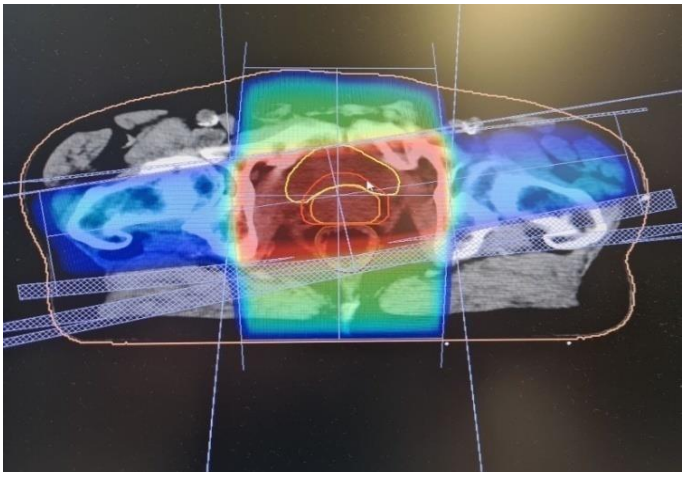

Figure 1. Treatment plan for 3DCRT technique in prostate cancer patient. Source: Institute for Oncology and Radiology of Serbia.

Radiation toxicity in patients with prostate cancer

After treating prostate cancer by radiotherapy, 5% to 10% of these patients may experience severe side effects affecting their quality of life (10, 11). Radiotoxicity may manifest in the lower gastrointestinal (GI) tract by damaging of rectum or bowel and genitourinary (GU) tract causing symptoms by urethral, bladder or prostate damage (12).

Inflammation underlies acute side effects, while tissue fibrosis is the main cause of late side effects from radiotherapy (12, 13). Acute toxicity is a temporary condition that affects high-turnover tissues, causing dermatitis, cystitis, or diarrhea, during or shortly after its completion (14). In contrast, rectal bleeding, urinary morbidity, erectile dysfunction and fibrosis are examples of late toxicity that can persist for a lifetime after treatment (15).

In contrast to acute radiotoxicity, which manifests within 120 days of the beginning of radiation therapy, late radiation morbidity is defined as side effects evaluated 120 days after the beginning of the treatment (16, 17). Acute toxicities were defined by some authors as those happening during the course of the treatment or within the initial 90 days following its finalization, whereas late side effects were considered as those appearing more than 90 days following the end of the RT or beginning acutely and persisting for more than 90 days following the treatment (18). Other authors classify GI and GU radiotoxicity into acute/sub-acute reactions, which occur during the course of radiotherapy and within 3 - 6 months after its completion, while late complications could start 6 months after radiotherapy and may last for years (19).

Although a number of protocols are used in clinical practice for grading acute and late radiotoxicity, a uniform system hasn't been established yet. The most commonly used schemes for assessing side effects are the European Organization for Research and Treatment of Cancer (EORTC) and Radiation Therapy Oncology Group (RTOG) a part of which is shown in **table 1** and **2** (20).

Since the majority of prostate cancer patients with localized disease at diagnosis have indolent tumors with a five-year survival rate of almost 100%, complications developed due to radiotherapy treatment may impair their quality of life (21).

Numerous trials have shown the value of dose escalation for control of tumor (22, 23). The possibility of complications of healthy surrounding tissues rises as the radiation dose is increased.

Therefore, during radiotherapy planning, it is necessary to follow dose constraints for healthy tissues using Quantitative Analysis of Normal Tissue Effects in the Clinic (QUANTEC) (24).

However, there are patients with satisfactory dosimetric parameters who develop radiation toxicity, as well

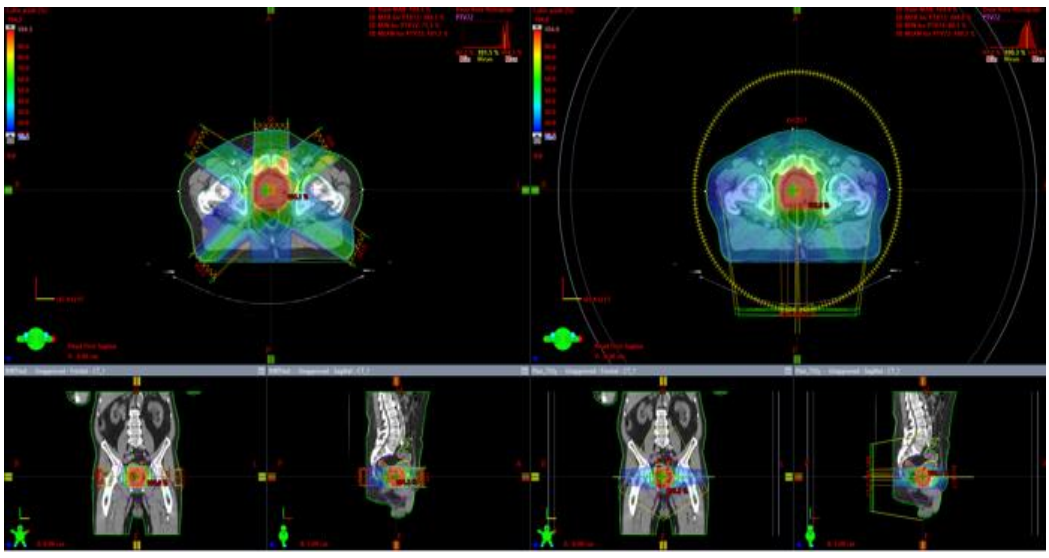

Figure 2. Treatment plan for IMRT and VMAT technique in prostate cancer patient. Source: Institute for Oncology and Radiology of Serbia.

Table 1. RTOG acute radiation toxicity criteria.

Tissue	Grade 1	Grade 2	Grade 3	Grade 4
Lower GI	Increased frequency or change in quality of bowel habits not requiring medication/ rectal discomfort not requiring analgesics	Diarrhea requiring parasympatholytic drugs/ mucous discharge not necessitating sanitary pads/rectal or abdominal pain requiring analgesics	Diarrhea requiring parenteral support/severe mucous or blood discharge necessitating sanitary pads/abdominal distention (flat plate radiograph demonstrates distended bowel loops)	Acute or subacute obstruction, fistula or perforation; GI bleeding requiring transfusion; abdominal pain or tenesmus requiring tube decompression or bowel diversion
Genitourinary tract	Frequency of urination or nocturia twice pretreatment habit/dysuria, urgency not requiring medication	Frequency of urination or nocturia that is less frequent than every hour. Dysuria, urgency, bladder spasm requiring local anesthetic	Frequency with urgency and nocturia hourly or more frequently/dysuria, pelvis pain or bladder spasm requiring analgetics	Hematuria requiring transfusion/acute bladder obstruction, ulceration, or necrosis

Table 2. RTOG late radiation toxicity criteria.

Tissue	Grade 1	Grade 2	Grade 3	Grade 4
Lower GI tract	Mild diarrhea; mild cramping; bowel movement 5 times daily; slight rectal discharge or bleeding	Moderate diarrhea and colic; bowel movement > 5 times daily; excessive rectal mucus or intermittent bleeding	Obstruction or bleeding, requiring surgery	Necrosis / Perforation Fistula
Genitourinary tract	Frequency during day 0.5 - 1 h; nocturia 2 - 3/ night; slight dysuria or microscopic hematuria requiring no medication; slight epithelial atrophy, minor telangiectasia; bladder capacity > 300 cc^3	Frequency during day 1 - 2 h; nocturia 4 - 6 / night; moderate dysuria or intermittent (mild, moderate) hematuria requiring medication; moderate telangiectasia; bladder capacity 150 - 300 cc^3	Frequency during day > 2 h; nocturia > 6/night; severe dysuria/frequent (severe) hematuria/severe telangiectasia; bladder capacity 100 - 150 cc^3, benign urethral strictures requiring a TURP, dilatation, suprapubic or permanent catheter	Necrosis; severe hemorrhagic cystitis; bladder capacity < 100 cc^3

as those with less favorable ones who develop toxicity less frequently. As a result, other parameters that can be utilized for the prediction of the development of radiotoxicity must be determined. It is considered that the principal mechanisms of normal tissue toxicity induced by radiotherapy are based on the depletion of tissue stem and progenitor cells as well as injury to vascular endothelial microvessels. The recovery and repopulation of stromal stem cells are considered to be chronically hampered by long-lived free radicals, reactive oxygen species or pro-inflammatory molecules with consequent progressive radiation-induced damage (25). Clinicians should be able to assess occurring of GI and GU morbidity prior to treatment with identifying variables that should be changed with the aim of reducing of the development of side effects.

Prediction models that take into account additional parameters to identify patients most susceptible to developing toxicity may serve as essential factors toward personalized radiotherapy. The main objectives are morbidity reduction and life-quality improvement (26).

Toxicity of the GI tract may vary from symptoms such as a slight increase in the frequency of bowel movement to much more serious issues like rectal bleeding, pain, or fistula. Inflammation in response to radiotherapy treatment characterizes the acute injury, whereas sclerosis and fibrosis characterize the late phase (27). Numerous symptoms, such as urinary frequency, hematuria, dysuria, obstruction and stricture, or incontinence, are associated with urinary toxicity. Vascular ischemia, cellular destruction, and edema, lead to fibroblast proliferation and bladder smooth muscle depletion, reducing bladder capacity and obstructing the urethral lumen (19).

Maintaining a patient's quality of life is a crucial factor affecting therapy decisions, since prostate cancer is a highly curable disease and has an expected long survival time (28).

The factors that affect radiosensitivity are individual, clinical characteristics and dose-volume effects, but the existing and known factors are not sufficient for understanding of the development of radiotoxicity. One of the challenges of radiotherapy is the identification of biomarkers associated with individual radiosensitivity. Changes in the cytokine levels could also be connected with the occurrence of acute gastrointestinal and genitourinary toxicity (29, 30). Other biological factors, such as gene expression levels, miRNA levels such as miR-21/146a/155 levels, and single nucleotide polymorphisms (SNPs) in transforming growth factor beta 1 (*TGFB1*) gene, can also cause the development of radiotoxicity (31, 32). These findings suggest that the determination of multiple biological parameters should be conducted in additional research to estimate individual radiosensitivity.

Cytokine role in the development of radiation toxicity

Soon after exposure of tissues to radiation therapy, the release of cytokines is triggered in the irradiated cells with the development of an inflammatory response and potential tissue damage. The goal of this work is to summarize the results of contemporary research in which the connection between the occurrence of acute radiation toxicity and changes in the cytokines levels in the serum during radiotherapy were examined.

Since it is suggested that inflammation is the basis of the development of acute radiation toxicity, it stands to reason that inflammatory factors, such as cytokines, could play a major part in the occurrence of GI and GU toxicity.

Therefore, determining cytokines levels during radiotherapy can have clinical value in predicting the radiosensitivity of healthy tissues with the aim of having a personalized approach to radiotherapy.

Determination of serum cytokine concentrations in prostate cancer patients is performed by ELISA test before, during and after the end of radiotherapy.

Among a number of cytokines that are wildly produced right after radiation exposure, Interleukin 1 (IL-1), interleukin 6 (IL-6), tumor necrosis factor (TNF), and transforming growth factor beta 1 (TGF-β1) are significant cytokines involved in the response of skin, lung and brain (25).

Rubin et al. suggested the association between toxicity caused by radiotherapy and cytokine levels. They concluded that after lung irradiation in C57BL/6 mouse with a single sublethal dose of 5 Gy or a lethal dose of 12.5 Gy, there is an early activation and maintenance of the cytokine cascade, primarily IL-1α and TGF-β1, and subsequent inflammation with the onset of pneumonitis and then after fibrosis (33). Stanojkovic et al. investigated the association of cytokines such as IL-1, TGF-β1, IL-2, IL-6, and interferon-gamma (IFN-γ) with the appearance of GI and GU radiotoxicity. They conducted a study including 44 patients treated with definitive or postoperative radiotherapy with doses of 72 Gy and 66 Gy, respectively (34). The most prominent cytokines associated with the development of GI and GU radiotoxicity in prostate cancer patients are shown in **figure 3**.

Numerous studies have shown significant changes in cytokine levels in serum and plasma after radiotherapy compared to those before RT treatment (35,36).

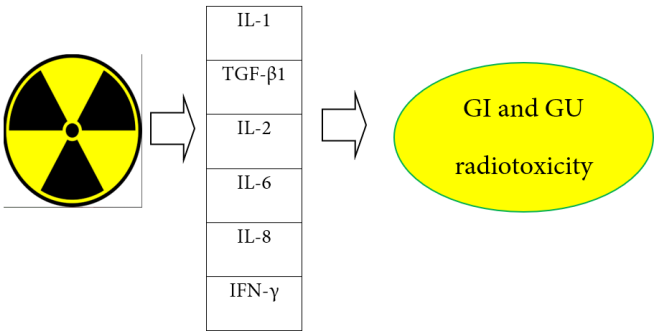

Figure 3. Cytokines associated with GI and GU radiotoxicity in prostate cancer patients.

Differential cytokine expression profiles measured before and during radiotherapy can be a good potential method for identifying of patients' normal tissue radiosensitivity in order to develop a tailored radiotherapy regimen.

The TGF-β1, which is produced by endothelial and hematopoietic cells, is encoded by the *TGFB1* gene. TGF-β1 controls a variety of cell processes, including angiogenesis, differentiation, immune response, and embryonic development (37). There is proof that TGF-β1 serves as a crucial fibrosis mediator by stimulating fibroblasts to generate extracellular matrix and enlist inflammatory cells (38).

Even at low doses, radiation is one of the external factors recognized to activate TGF-β1 (39). All TGF-β1 levels have been linked with higher degrees of fibrosis following thoracic and abdominopelvic radiotherapy (40). The binding of TGF-β1 to type I/II receptor complexes results in the activation of SMAD proteins. It is suggested that activation of SMAD3 is associated with the development of radiation-induced fibrosis (41).

The results of various studies indicate that IL-6 and TGF-β1 could participate in the occurrence of acute genitourinary toxicity in prostate cancer patients.

Kopcalic et al. noticed that the circulating levels of IL-6 after the 25th radiotherapy fraction were significantly higher when compared to levels measured prior to the start of radiotherapy treatment which is shown in **table 3**. Also, they concluded that acute genitourinary toxicity grades were positively correlated with the IL-6 and the TGF-β1 levels measured in the 25th fraction (42).

Malisic et al. found that TGF-β1 C-509T heterozygote carriers (CT) cause a significantly lower rate of acute radiotoxicity compared to homozygote (CC plus TT) carriers in patients with prostate cancer (32).

Table 3. Serum concentrations of IL-6 and TGF-β1 in patients with prostate cancer before radiotherapy and after the 25th radiotherapy fraction (Kopcalic et al. (42)).

Cytokine	Before radiotherapy	After the 25th radiotherapy fraction	p
	Concentration (pg/ml) Median (minimum - maximum)	Concentration (pg/ml) Median (minimum - maximum)	
IL-6	4.6 (0.8 - 41.7)	6.3 (2.3 - 49.3)	**< 0.001**
TGF-β1	9152.8 (1600.3 - 37421.8)	8104.4 (2465.1 - 20718.9)	0.136

Literature data suggest that prior surgery could have a role in the development of radiotoxicity far from the surgical area through TGF-β1 (43). Additionally, it has been noted that surgery increases the expression of cytokines with the consequent development of GI and GU mucosa injuries (44,45).

Johnke et al. suggested that IL-6, TGF-β1 and IL-1β levels, significantly increase during radiotherapy treatment (46). Both IFN-γ and IL-6 concentrations were significantly elevated during IMRT, according to Christensen et al. who also noticed that acute GI and GU complications are significantly associated with increasing IL-2 and IL-1 levels, respectively, as shown in **figure 4** (29).

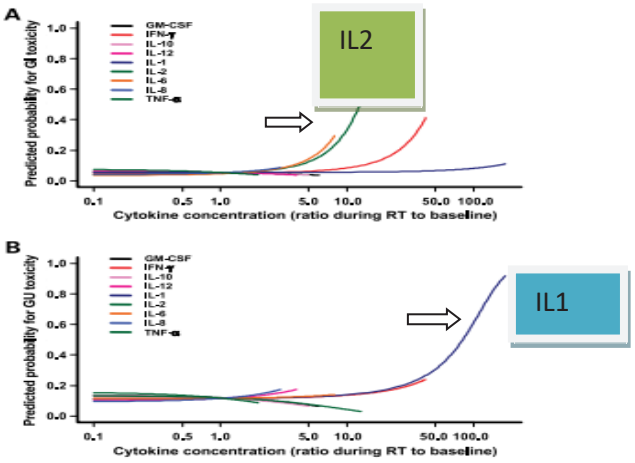

Figure 4. Predicted probability for GI and GU toxicity in relation to the level of cytokines (Christensen et al. (29)).

According to the literature, IL-6 is regarded as one of the most important immune markers for predicting the RT-induced toxicity of normal tissues (47,48). It has been suggested that patients undergoing definitive radiotherapy have substantially greater levels of IL-6 and IFN-γ than those who are treated with postoperative radiotherapy treatment (34). Taking into account that prostate cancer cells produce IL-6, which could have a role in the inflammatory response to radiotherapy, this finding for this pro-inflammatory cytokine was expected (49,50). Tazaki et al. showed an increased IFN-γ serum level in prostate cancer patients (51).

It is supposed that higher levels of IL-6 contribute to a more aggressive tumor and treatment resistance. Therefore, therapeutic modulation of this cytokine may improve tumor radiotherapy sensitivity (52).

Another cytokine - TNF, produced primarily by activated macrophages but also by other hematopoietic and non-hematopoietic cells - plays a role in the development of acute and chronic inflammation. An increase in TNF level along with IL-1 causes significant signs of acute skin reaction, while IL-1 seems to be crucial in the inflammatory response (53). The inhibitors have been shown in pre-clinical studies to reduce radiation-induced skin damage in mice (54).

A cytokine storm, which is the overemphasized immune system's activity in response to outside stimuli like infections, immunotherapy, and therapeutic interventions such as radiotherapy, can lead to the production of cytokines (55). The concept of "cytokine storm" was initially presented by Ferrara JL, who suggested that inflammatory cytokines may play a role in the acute graft versus host disease (GVHD) process (56). According to Hill GR and colleagues, the majority of the clinical manifestations of GVHD are caused by T cells' and other inflammatory cells' dysregulated cytokine production.

The GI tract is essential to the development of the cytokine storm since the GI tract increases the translocation of inflammatory stimuli such as endotoxin, resulting in more inflammation and further GI tract injury (57).

Radiotherapy (RT) is considered to have the potential to induce cytokine storm with several mechanisms of immune system activation. Damage-associated molecular patterns (DAMPs) induced by RT, either as exposure on the cell surface or released passively or actively by cancer cells, may interact with tumor cell pattern recognition receptors (PRRs) inducing immunogenic cell death of cancer cells (58). Radiation therapy may also cause chemokine secretion, which induces different inflammatory immune cells into the tumor microenvironment. In addition, radiotherapy may cause signal pathway activation based on the tumor neoantigens, with a consequent increase in interferon expression.

All immune activation effects mentioned above could cause a cytokine storm with releasing different kinds of cytokines.

Conclusion

Literature data suggest that changes in levels of circulating cytokines during radiotherapy might be one of the crucial indicators for the development of radiotoxicity.

Based on the above findings, it can be concluded that there is an association between radiation therapy, the development of inflammation, and the occurrence of radiation toxicity. However, further research with the aim of adequate stratification of patients and further strategies for the development of an individualized approach to radiotherapy to protect or mitigate radiation tissue damage is required.

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