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Thao-Nguyen Pham, Julie Coupey, Viktoriia Ivanova, Juliette Thariat, Samuel Valable. Differential plasma cytokine variation following X-ray or proton brain irradiation using machine-learning approaches. *Cancer/Radiothérapie*, 2024, 28 (5), pp.474-483. 10.1016/j.canrad.2024.08.001 . hal-04705579

HAL Id: hal-04705579

<https://normandie-univ.hal.science/hal-04705579v1>

Submitted on 23 Sep 2024

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Original article

Differential plasma cytokine variation following X-ray or proton brain irradiation using machine-learning approaches

Étude des variations différentielles des cytokines plasmatiques après irradiation cérébrale par rayons X ou protons à l'aide des approches d'apprentissage automatique

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INFO ARTICLE

Historique de l'article :
Reçu le 29 mars 2024
Accepté le 19 août 2024

Keywords :
Brain irradiation
Cytokine
Inflammation
Mouse model
Proton radiation
protonique

ABSTRACT

Purpose. – X-ray and proton irradiation have been reported to induce distinct modifications in cytokine expression in vitro and in vivo, suggesting a dissimilar inflammatory response between X-rays and protons. We aimed to investigate the differences in cytokine profiles early following fractionated brain irradiation with X-rays or protons and their relationship with leukocyte subpopulations in rodents.

Materials and methods. – Our study utilized data from 80 tumor-free mice subjected to X-ray or proton brain irradiation in four fractions of 2.5 Gy. Sixteen non-irradiated mice were used as the controls. Blood was collected 12 h postirradiation to examine the profile of 13 cytokines. Correlation analysis, principal component analysis (PCA), and tree-based modeling were used to investigate the relationship between cytokine levels and leukocyte subpopulation variations following irradiation in the blood.

Results. – Regardless of the irradiation type, brain irradiation resulted in a notable elevation in the plasma levels of IFN- γ and MCP-1. The use of either X-ray or proton beam had differential effect on plasma cytokine levels following brain irradiation. Specifically, X-ray irradiation was associated with significantly increased plasma levels of IFN- β , IL-12p70, and IL-23, along with a decreased level of IL-1 α , in comparison to proton irradiation. Correlation analysis revealed distinct cytokine regulatory patterns between X-ray and proton brain irradiation. PCA highlighted the association of MCP-1, IL-6, TNF- α , IL-17A, and IFN- γ with neutrophils, monocytes, and naïve T-cells following X-ray irradiation. TNF- α and IL-23 levels correlated with naïve CD4⁺-cells following proton irradiation. Tree-based models demonstrated that high TNF- α level resulted in an increase in naïve T-cells, neutrophils, and monocytes, whereas low IL-6 level was associated with decreases in these cell counts.

Conclusion. – Our findings revealed distinct inflammatory responses induced by X-ray irradiation in contrast to proton brain irradiation, as demonstrated by the differential regulation of cytokines in the bloodstream. Moreover, the study highlighted the association between specific cytokine levels and various leukocyte subpopulations. Further investigation is essential to accurately determine the impact of proton and X-ray brain irradiation on the inflammatory response and the efficacy of radiotherapy treatment.

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<https://doi.org/10.1016/j.canrad.2024.08.001>

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Pour citer cet article : T.-N. Pham, J. Coupey, V. Ivanova et al., Differential plasma cytokine variation following X-ray or proton brain irradiation using machine-learning approaches, Cancer Radiother, <https://doi.org/10.1016/j.canrad.2024.08.001>

R É S U M É

Mots clés :
Irradiation cérébrale
Cytokine
Inflammation
Radiation protonique

Objectif de l'étude. – L'irradiation par protons ou rayons X produit des modifications distinctes de l'expression des cytokines in vitro et in vivo, suggérant que les protons pourraient induire une réponse inflammatoire différente de celle des rayons X. Nous visons à étudier la différence dans les profils de cytokines suite à une irradiation cérébrale par rayons X ou protons et leurs associations avec les sous-populations de leucocytes.

Matériels et méthodes. – Notre étude a utilisé des données de 80 souris soumises à une irradiation cérébrale par rayons X ou protons, administrée en quatre fractions de 2,5 Gy. Du sang a été collecté 12 h après l'irradiation pour examiner le profil de 13 cytokines. L'analyse de corrélation, l'analyse en composantes principales (PCA), des apprentissages par arbre de décision et forêt aléatoire ont été utilisés pour enquêter sur la relation entre les cytokines/chémokines et les variations des sous-populations de leucocytes après l'irradiation cérébrale.

Résultats. – L'irradiation cérébrale a entraîné une élévation des niveaux plasmatiques d'IFN- γ et de MCP-1 indépendamment du type d'irradiation. L'irradiation par rayons X était associée à des niveaux plasmatiques significativement accrus d'IFN- β , d'IL-12p70 et d'IL-23, et réduit d'IL-1 α , par rapport à l'irradiation par protons. L'analyse de corrélation a révélé que des schémas régulateurs distincts des cytokines émergeaient entre le rayon X et le proton. La PCA a mis en évidence l'association de MCP-1, IL-6, TNF- α , IL-17A et IFN- γ avec des variations dans les neutrophiles, monocytes et cellules T naïves sous l'irradiation par rayons X. Les niveaux de TNF- α et IL-23 étaient corrélés avec les cellules CD4+ naïves sous l'irradiation par protons. Des apprentissages par arbre de décision et forêt aléatoire ont démontré que le niveau élevé de TNF- α entraînait des augmentations dans les cellules T naïves, les neutrophiles et les monocytes, tandis qu'un niveau faible d'IL-6 était associé à des diminutions dans ces cellules.

Conclusion. – Nos résultats ont mis en lumière les réponses inflammatoires distinctes élicitées par l'irradiation cérébrale par rayons X par rapport aux protons, comme en témoigne la régulation différentielle des cytokines. En outre, notre étude a élucidé les corrélations de certains cytokines spécifiques avec les sous-populations de leucocytes.

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1. Abbreviations

IL	Interleukin
TNF	Tumor necrosis factor
INF	Interferon
MCP	Monocyte chemoattractant protein
GM-CSF	Granulocyte-macrophage colony-stimulating factor
MFI	Mean fluorescent intensity
PCA	Principal component analysis
MSE	Mean squared error
%IncMSE	Mean squared error increment
PC	Principal component
RMSE	Root mean squared error

2. Introduction

Radiotherapy serves as one of the most commonly used treatment strategies in multiple types of cancer, including brain cancer. The role of cytokines in the immune response following cancer treatment including radiotherapy involves a complex interplay of systemic effects [1]. Prior studies have implicated cytokines in the initiation and maintenance of radiation-induced toxicity [2,3]. Conversely, cytokine release has been posited to contribute to the radiation-induced abscopal effect by activating the innate immune system and inducing a pro-inflammatory state [4]. Conventional X-ray irradiation has been shown to significantly increase the expression of key pro-inflammatory cytokines, such as interleukin (IL)-6, IL-1 β , and tumor necrosis factor (TNF)- α , not only in locally irradiated tissues but also systemically, highlighting the broader implications of local irradiation on systemic inflammation [5].

In the context of brain irradiation, whole-brain radiation therapy has been associated with neuroinflammation and an increase in pro-inflammatory cytokines in the blood, such as TNF- α , along with variations in leukocyte subpopulations. This system-

mic response has gained attention, especially with the promising combination of radiotherapy and immunotherapy for cancer treatment. Proton therapy, with its more precise dose distribution, poses a lower risk to surrounding normal tissues compared to conventional photon therapy and might be associated with differential effects on cytokines [6,7]. The biological responses to proton and photon radiation differ at the cellular level, with certain genes involved in angiogenesis and inflammation, such as vascular endothelial growth factor A, IL-6, and IL-8. These cytokines were upregulated following photon irradiation but downregulated after proton irradiation, indicating distinct transcriptional responses to different types of irradiation [8,9]. This is further supported by in vitro studies showing the differential expression of pro-inflammatory factors, such as IL-6 and IL-8, between photon- and proton-irradiated cells, further underscoring the intricate relationship between irradiation type and immune response [7,8,10]. Our previous analysis revealed disparities in the levels and interactions of leukocyte subpopulations following either X-ray or proton irradiation of the brain, indicating a differential systemic immune response to various radiation beams [11,12].

Cytokines are normally expressed as a complex network with multiple correlations rather than as independent individual [13]. In fact, one cytokine can compensate for another, and multiple cytokines may be simultaneously involved in a given biological function [14]. Machine learning with multivariate methods is a useful tool for analyzing such data. The application of machine-learning techniques in analyzing cytokine data has provided new insights into the association between cytokine profiles and systemic effects under various conditions [15,16].

The hypothesis for the present study was that exposure to either X-rays or protons following brain irradiation would result in distinct systemic cytokine profiles, which in turn could modify the variation of leukocyte subpopulations following brain irradiation. This study aimed to investigate the differences in cytokine profiles early after fractionated brain irradiation in rodents using X-rays or

protons. The impact of cytokine profiles on leukocyte subpopulations was investigated using machine-learning approaches.

3. Materials and methods

3.1. Analysis of plasma cytokine variation following brain irradiation

Two preclinical experiments were conducted to investigate the effects of brain irradiation on leukocyte populations in 96 healthy tumor-free C57BL/6 adult mice. In each experiment, 40 mice received a total dose of 10 Gy administered in four twice-daily fractions (2.5 Gy) over two consecutive days, and eight mice were used as controls.

Blood samples were collected at 12 h after the last irradiated fractions. Mice that were not subjected to irradiation were used as controls. Details of the experimental design have been described previously [11]. Cytokine analysis protocol is described in [Supplementary information](#). Cytokine levels were quantified by flow cytometry using the LEGENDplex™ kit (BioLegend). The plasma levels of 13 cytokines were analyzed. The cytokine panel included interleukin (IL)-1 α , IL-1 β , IL-6, IL-10, IL-12(p70), IL-17A, IL-23, IL-27, interferon (IFN)- β , IFN- γ , tumor necrosis factor (TNF)- α , monocyte chemoattractant protein (MCP)-1, and granulocyte-macrophage colony-stimulating factor (GM-CSF). Mean fluorescence intensity (MFI) was used for analysis. MFI was scaled relative to the mean MFI value of the control mice in each study. For details of the cytokine analysis protocol, see [Supplementary information](#). Our previous studies analyzed and reported variations in leukocyte subpopulations following brain irradiation with either X-rays or protons, under identical experimental conditions and protocols [11,12].

3.2. Analysis of plasma cytokine variation in relation to leukocyte subpopulation level in the blood following brain irradiation

3.2.1. Correlation between cytokine level following irradiation

Pairwise Pearson correlations between 156 pairs of cytokines among the 13 cytokines were calculated. To investigate the change in correlation following brain irradiation in each study, the change in correlation was calculated as follows:

$$\Delta R(X_i, X_j) = R_T(X_i, X_j) - R_C(X_i, X_j)$$

where $\Delta R(X_i, X_j)$ is the difference in the Pearson correlation coefficients of cytokines X_i and X_j between the irradiated and control groups.

3.2.2. Principal component analysis (PCA)

PCA was applied to group related cytokine levels following brain irradiation into specific components. PCA was performed using the *FactoMineR* package (for the analysis) and *factoextra* package (for data visualization) in the R software [17]. Components that explained more than 10% of the data variation were selected.

The relationship between each component and leukocyte subpopulations (T-cells, B-cells, neutrophils, and monocytes), and T-cells subpopulations (naïve T CD4⁺ cells, naïve T-CD8⁺ cells, memory T-CD4⁺ cells, memory T-CD8⁺ cells) levels early after irradiation were analyzed.

3.2.3. Tree-based modeling

A tree-based approach was used to analyze any existence of hierarchical interactions between plasma cytokine levels and leukocyte subpopulations following brain irradiation. This study utilized both decision tree and random forest models to analyze

the impact of cytokine levels on immune cell variations following brain irradiation with X-rays.

The tree was built using the mean squared error (MSE) for splitting node decisions. Specifically, the node-splitting strategy minimizes the MSE. The splitting process was stopped when there was no further possible splitting. This procedure was performed using the *rpart* package version 4.1.19 [18]. A post-pruning process was performed to remove nonsignificant branches.

The random forest model was used to search for relevant contribution of plasma cytokine level to leukocyte subpopulations following irradiation. Parameter importance ranking was obtained using random forest regression in terms of the MSE increment (%IncMSE) when imputing the parameters from the model. The stronger impact of the parameters was reflected by the higher %IncMSE.

4. Results

4.1. The change of plasma cytokine following brain irradiation

We first investigated changes in the plasma levels of cytokines following brain irradiation. Brain irradiation independently on the type of irradiation, led to a significant increase in plasma levels of IFN- γ and MCP-1 ($P = 0.01$). No significant changes were observed in the plasma levels of GM-CSF, IFN- β , IL-10, IL-12p70, IL-17A, IL-1 β , IL-23, IL-27, IL-6, and TNF- α ($P > 0.1$; [Fig. 1A](#)).

Differential patterns of cytokine variation were revealed when the data were stratified according to the type of irradiation beam. X-ray brain irradiation was associated with a significant reduction in IL-1 α levels ($P < 0.01$) compared to those in control mice. In contrast, proton irradiation did not result in significant changes in IL-1 α levels compared to the controls. Following proton brain irradiation, there was an increase in the plasma levels of MCP-1 ($P = 0.01$) relative to control mice ([Fig. 1B](#), for more details see [Supplementary Fig. 1](#)). X-ray irradiation led to significantly higher plasma levels of IFN- β , IL-12p70, and IL-23 ($P < 0.01$) and a lower level of IL-1 α ($P < 0.01$) compared to proton irradiation ([Fig. 1B](#)).

4.2. Plasma cytokine variation in relation to leukocyte subpopulation level in the blood following brain irradiation

We then aimed to investigate the relationship between the variation in plasma cytokine levels and circulating leukocyte subpopulations following brain irradiation. In our previous studies with identical mice, radiation-induced lymphopenia occurred in all lymphoid subpopulations following X-ray irradiation, a phenomenon that was not observed with proton irradiation [11,12]. At a summary level, there was an increase in neutrophil count after proton irradiation, a change not evidenced after X-rays. There was no statistically significant change in monocyte counts following both X-ray and proton brain irradiation. However, at an individual level, brain irradiation with both X-rays and protons resulted in high inter-individual variability between individuals in neutrophil and monocyte counts. In T-cell subpopulations, significant reductions in both naïve and memory T-CD8⁺ cells were observed after X-ray irradiation, whereas no significant change was noted in T-CD4⁺ cells. Similarly, proton irradiation did not result in statistically significant changes in the levels of naïve and memory T-cells.

4.2.1. Modification in correlation between cytokine level following irradiation

We aimed to uncover the differential correlations between plasma cytokine levels resulting from brain irradiation with either X-rays or protons. Distinct regulatory patterns in plasma cytokine levels following brain irradiation were observed between X-rays

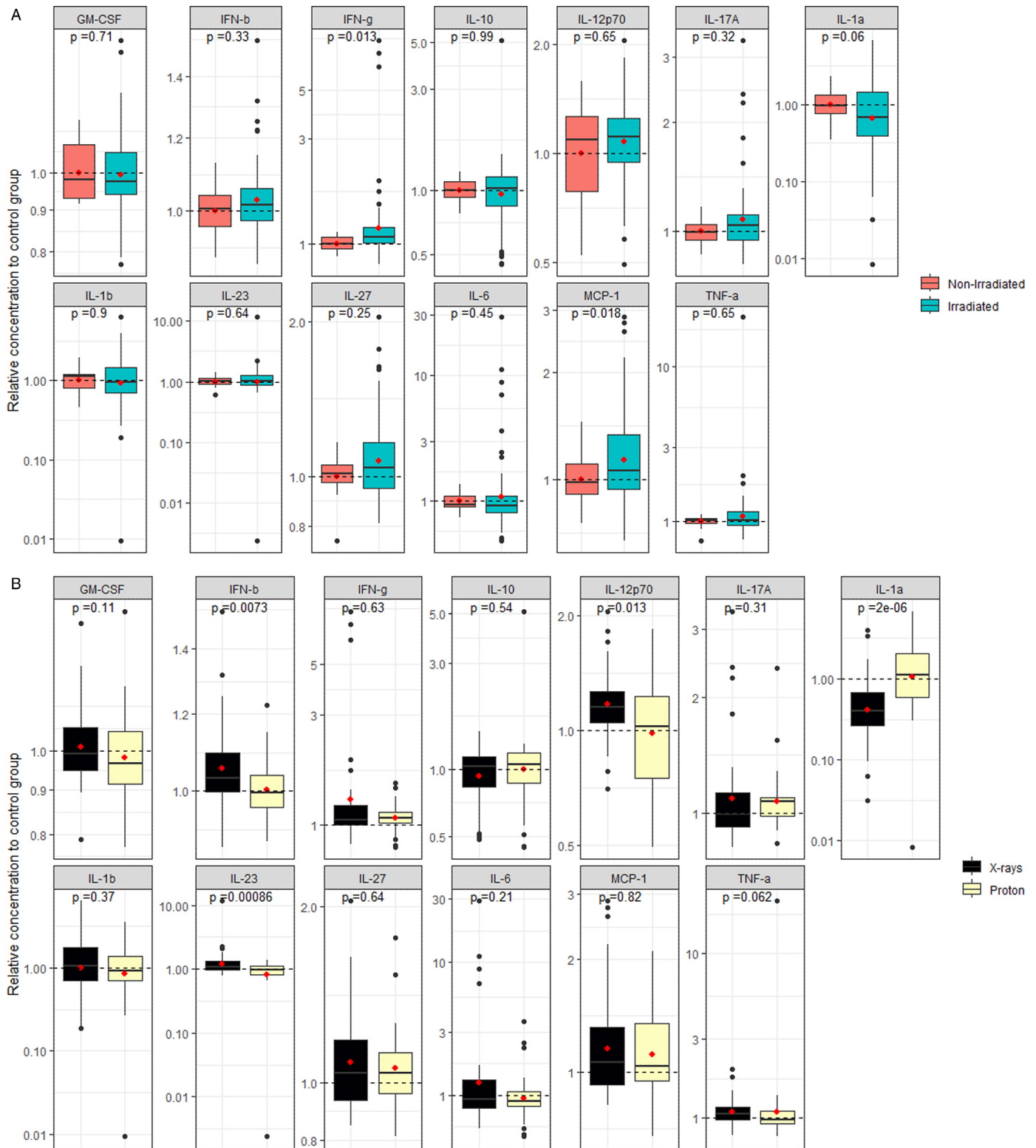


Fig. 1. Cytokine variation following brain irradiation: changes in mean fluorescence intensity of plasma cytokine levels relative to the control group following brain irradiation. A. Comparison between the control (non-irradiated) and treated (irradiated) mouse groups. B. Comparison between X-ray and proton irradiation of the brain. Comparisons were performed using the non-parametric Wilcoxon test. GM-CSF: granulocyte-macrophage colony-stimulating factor; IL: interleukin; INF: interferon; MCP: monocyte chemoattractant protein; TNF: tumor necrosis factor.

and protons, as evidenced by the differences in their correlation with the control group (Fig. 2).

Following proton irradiation, an increase was observed in the correlation coefficients between IL-1 β and a group of cytokines, including MCP-1, IL-6, IFN- β , and IL-10, when compared to control

mice. Conversely, X-ray exposure resulted in lower correlation coefficients between IL-1 β and IL-10, as well as between IL-1 β and IFN- β , relative to the control group. The correlation between IL-1 β , IL-6, and GM-CSF following X-ray brain irradiation showed negligible deviation from the control mice (Fig. 2A).

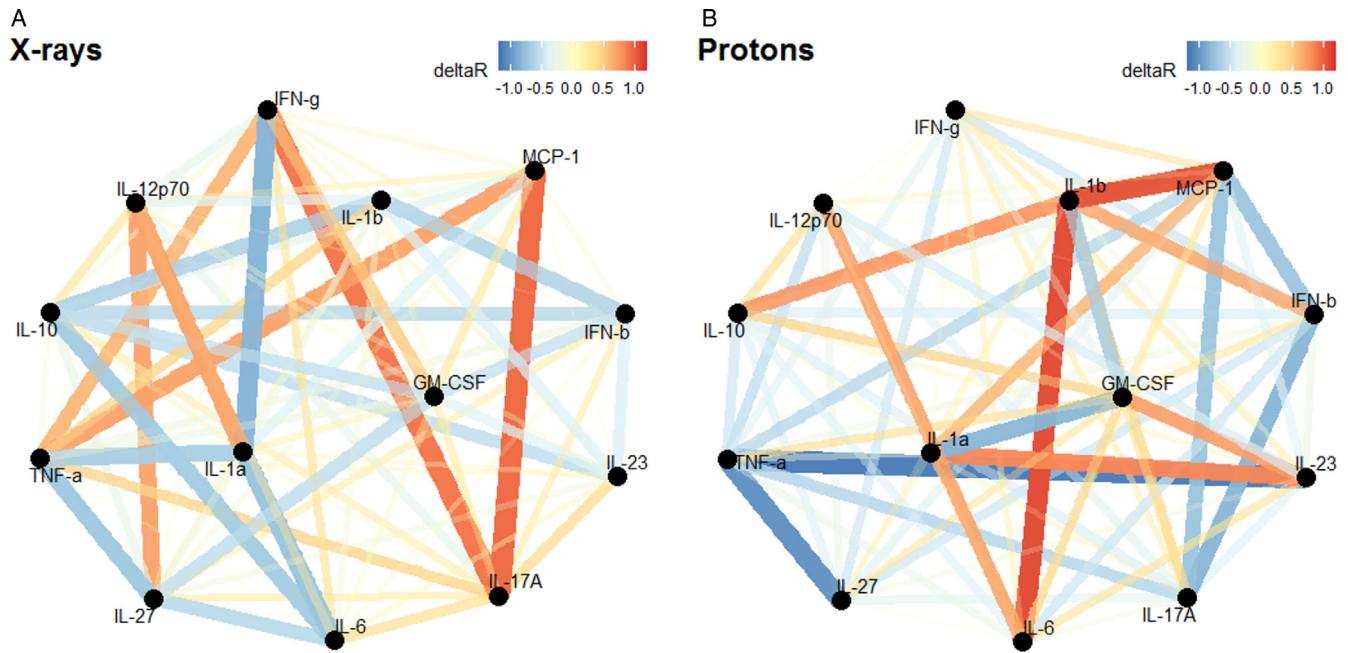


Fig. 2. Cytokine variation following brain irradiation: relationship between plasma cytokine levels following brain irradiation with X-rays (A) or protons (B) based on the change in the correlation coefficient between the irradiated and control groups. The black node represents the cytokine levels, and the edge represents the difference in correlation between the irradiated and control groups. Color represents the intensity of the difference in the correlation between the irradiated and control groups. GM-CSF: granulocyte-macrophage colony-stimulating factor; IL: interleukin; INF: interferon; MCP: monocyte chemoattractant protein; TNF: tumor necrosis factor.

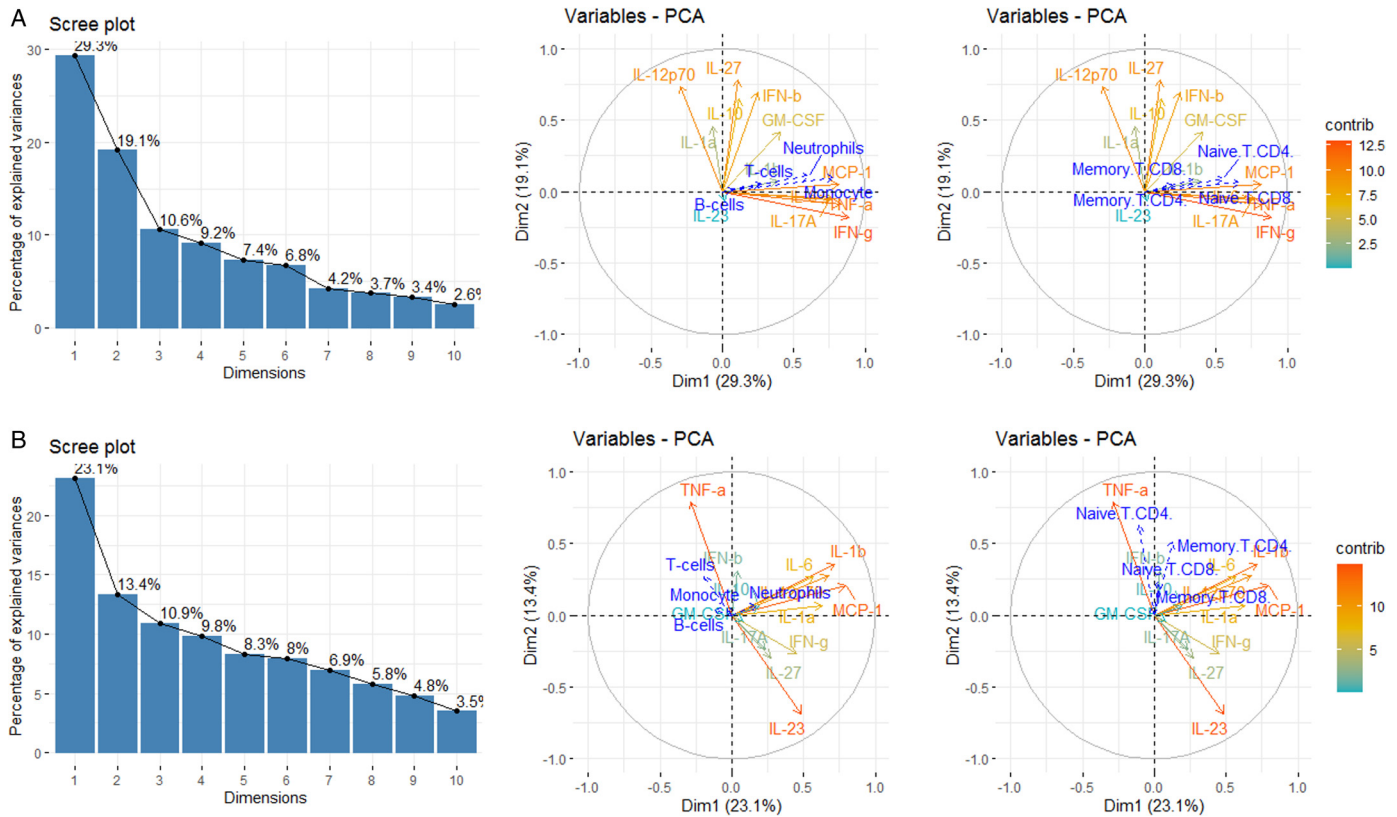


Fig. 3. Cytokine variation following brain irradiation: principal component analysis results of plasma cytokine variation following brain irradiation in relation to leukocyte and T-cell subpopulations following X-ray- (A) or proton- (B) brain irradiation. The blue arrow and text indicate the effects of independent variables. The legend “cos²” represents the contributions (on a scale of zero to one) of the variables to the two main principal components. GM-CSF: granulocyte-macrophage colony-stimulating factor; IL: interleukin; INF: interferon; MCP: monocyte chemoattractant protein; TNF: tumor necrosis factor.

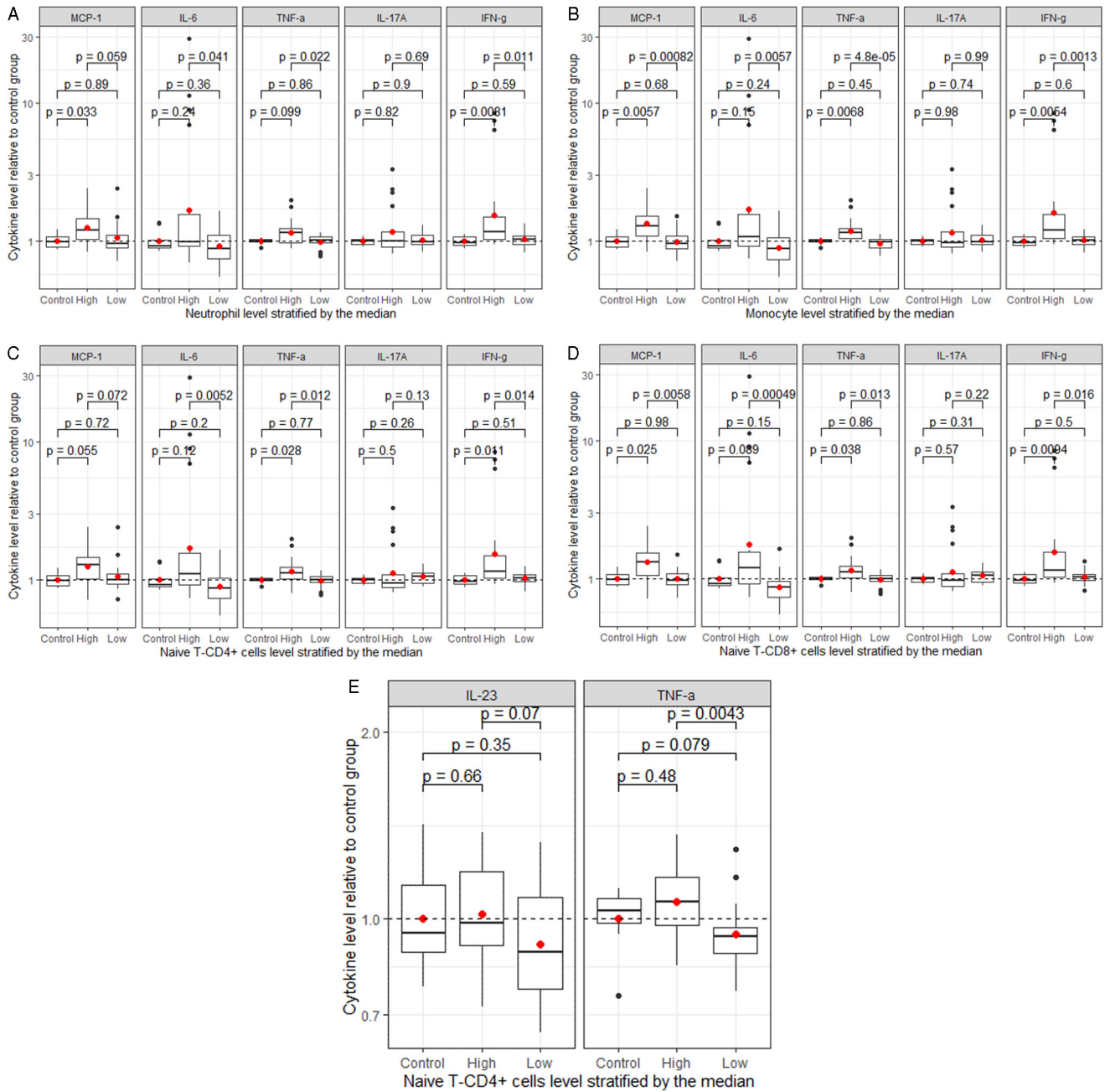


Fig. 4. Cytokine variation following brain irradiation: effect of MCP-1, IL-6, TNF- α , IL-17 α , and IFN- γ on myeloid cells (neutrophils and monocytes) and naïve T-cells (CD4+ and CD8+) following X-ray irradiation (A, B, C, D), and the effect of IL-23 and TNF- α on naïve T-CD4+ cells following proton irradiation (E). IL: interleukin; INF: interferon; MCP: monocyte chemoattractant protein; TNF: tumor necrosis factor.

Additionally, proton irradiation was associated with a reduction in the correlation between TNF- α and both IL-23 and IL-27, with the decrease in the correlation between TNF- α and IL-27 being more pronounced following X-ray irradiation. No significant change was noted in the correlation between TNF- α and IL-23 levels following X-ray exposure compared with controls (Fig. 2B).

X-ray treatment also led to an increased correlation between MCP-1 and IL-17A levels, a relationship that was diminished following proton irradiation relative to the controls. Furthermore, in the context of X-ray irradiation, there was a notable decrease in the correlation coefficient between IL-6 and both IL-10 and IL-27 compared to that in control mice, which was not observed in proton-treated mice.

4.2.2. Plasma cytokine variation in relation to leukocyte subpopulation level in the blood following brain irradiation

Given the substantial number of cytokines and their interrelationships, we aimed to reduce the dataset dimension with a smaller number of uncorrelated variables using PCA. Subsequently, we explored the influence of plasma cytokine levels on the variations in leukocyte subpopulations in response to brain irradiation.

Following X-ray irradiation, the first principal component (PC1) accounted for 29.3% of the variance and demonstrated significant correlations (correlation coefficient, $R > 0.5$, Fig. 3A) with MCP-1, IL-6, TNF- α , IL-17A, and IFN- γ . Myeloid cells (including neutrophils and monocytes) and naïve T-cells (both CD4+ and CD8+) were also positively correlated with PC1 (correlation coefficient, $R > 0.5$,

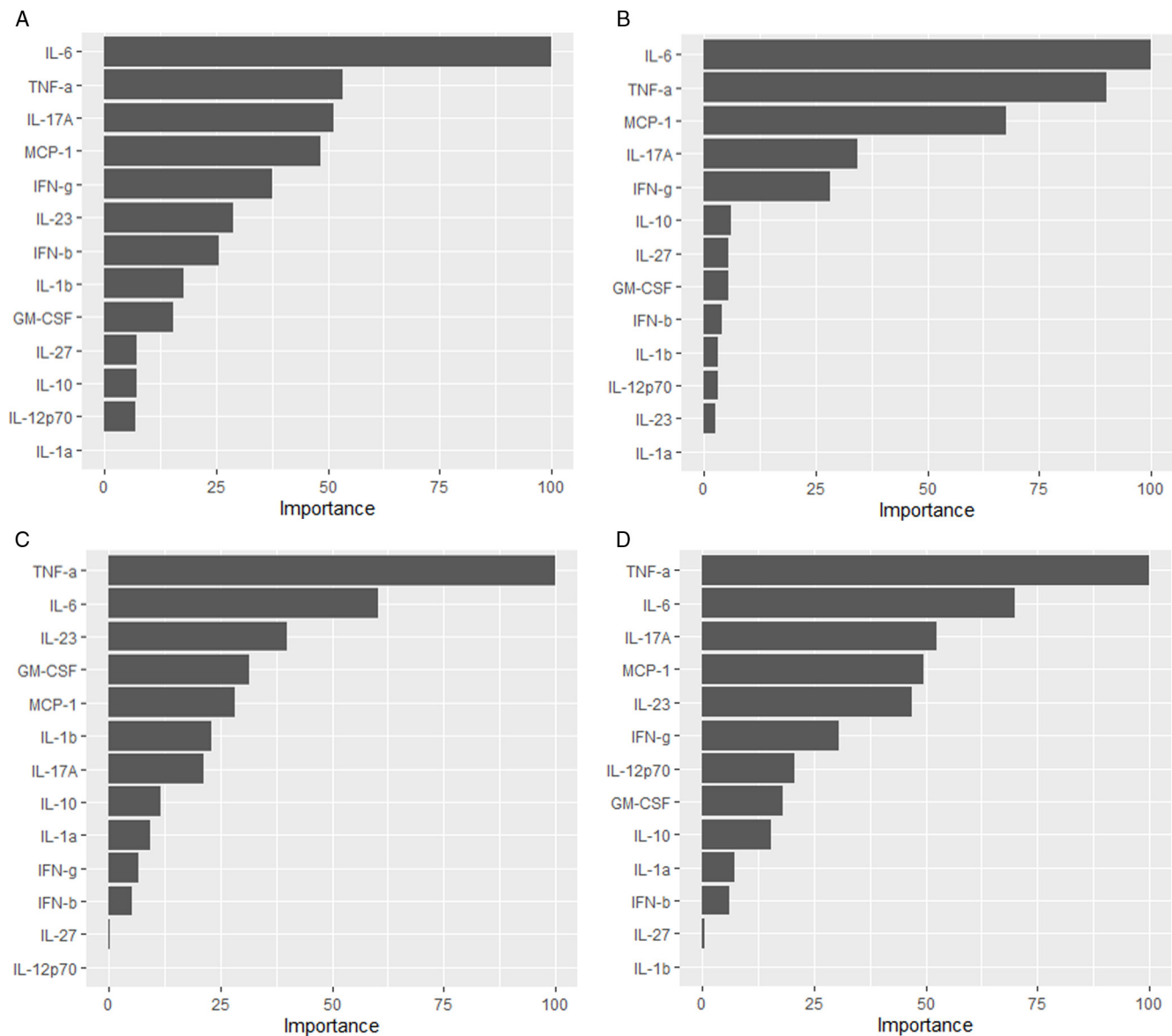


Fig. 5. Cytokine variation following brain irradiation: parameter importance according to the random forest model for neutrophils (A), monocytes (B), naïve T-CD4+ cells (C), and naïve T-CD8+ cells (D). The parameter importance ranged from 100% (the most important parameters to neutrophil, monocyte, naïve T CD4+ cell, or naïve T CD8+ cell count variation) to 0% (parameters with no contribution to neutrophil, monocyte, naïve T CD4+ cell, or naïve T CD8+ cell count variation). GM-CSF: granulocyte-macrophage colony-stimulating factor; IL: interleukin; INF: interferon; MCP: monocyte chemoattractant protein; TNF: tumor necrosis factor.

Fig. 3A). The second principal component (PC2) was responsible for 19.1% of the variance, showing strong correlations (>0.5) with IFN- β , IL-12p70, IL-27, and IL-10 levels. Notably, the leukocyte and T-cell subpopulations did not correlate with PC2 (Fig. 3A).

Based on the correlation between certain leukocyte subpopulations and cytokine levels in PC1, we investigated the effects of MCP-1, IL-6, TNF- α , IL-17A, and IFN- γ on myeloid cells (neutrophils and monocytes; Fig. 4A, B) and naïve T-cells (CD4+ and CD8+; Fig. 4C, D) following X-ray irradiation. Mice were categorized into groups with high or low levels of each cell line as determined by the median. High neutrophil levels were defined as those exceeding 103.08% of the neutrophil count in control mice, whereas low neutrophil levels fell below this threshold. High levels of neutrophils relative to the monocyte count were considered to be above 76.57% compared to the control mice, with low levels below this value. High counts of naïve T-CD4+ cells exceeded 67.64% of the control group's count, with low counts falling below this percentage. For naïve T-CD8+ cells, high levels were set above 37.53% and

low levels were defined as below this percentage. We noted a significant increase in the plasma concentrations of MCP-1 and IFN- γ in groups with high levels of neutrophils, monocytes, naïve T-CD4+ cells, and naïve T-CD8+ cells compared to groups with low levels and the control group (Fig. 4A, B, C, D). These groups also exhibited significantly higher plasma IL-6 and TNF- α levels.

In proton irradiation, PC1 explained 23.1% of the variance and was significantly correlated (correlation coefficient, $R > 0.5$, Fig. 3B) with MCP-1, IL-1 α , IL-1 β , IL-6, and IL-12p70, although it did not correlate with any leukocyte or T-cell subpopulations. PC2, which accounted for 13.4% of the variance, displayed significant correlations (correlation coefficient, $R > 0.5$) with TNF- α and a negative correlation (correlation coefficient, $R < -0.5$) with IL-23 (Fig. 3B). Naïve T-CD4+ cells were positively correlated with PC2 (correlation coefficient, $R > 0.5$, Fig. 3B).

We further analyzed the roles of IL-23 and TNF- α in naïve T-CD4+ levels following proton brain irradiation (Fig. 4E). High levels of naïve T-CD4+ cells were defined as those exceeding 120.70%

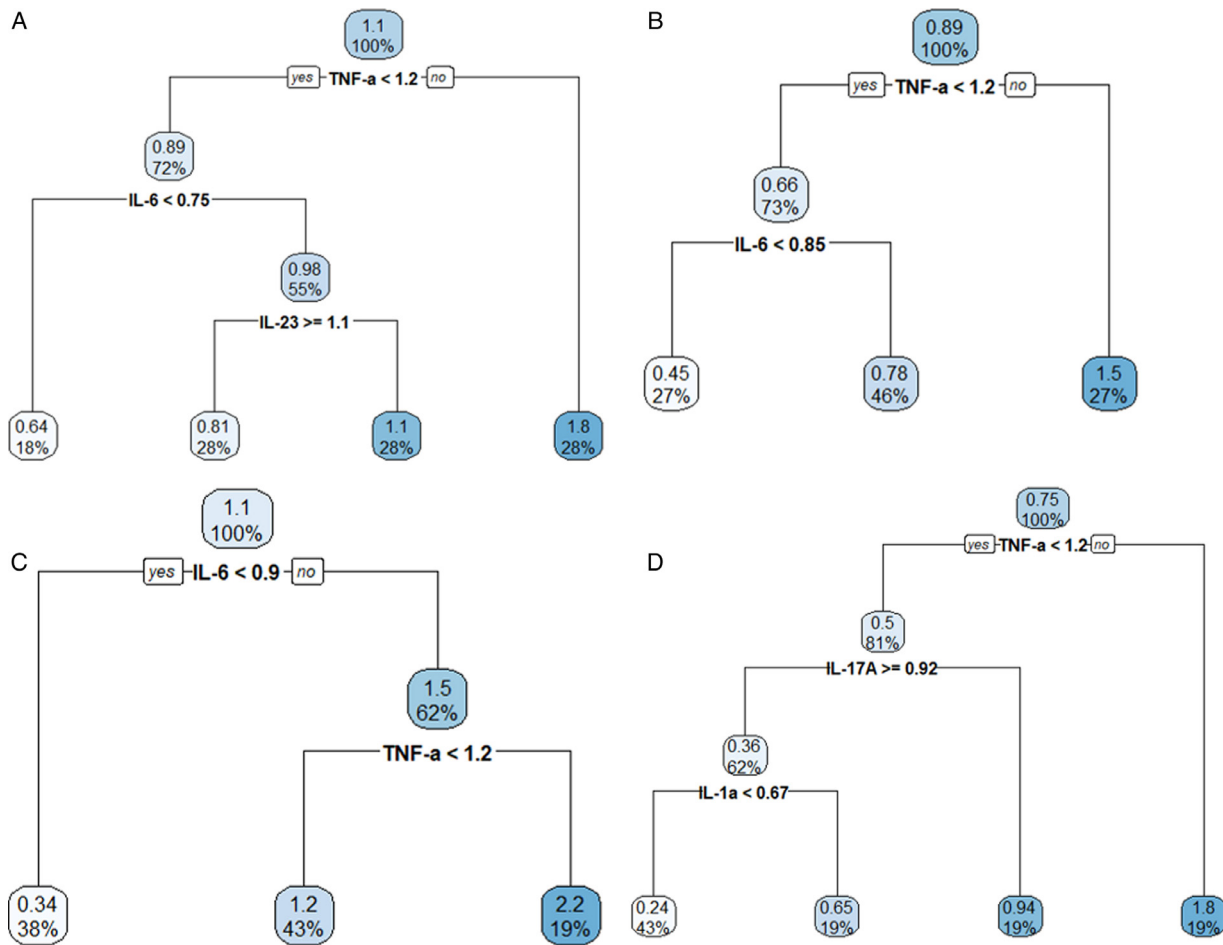


Fig. 6. Cytokine variation following brain irradiation: decision tree model of neutrophils (A), monocytes (B), naïve T-CD4+ cells (C), and naïve T-CD8+ cells (D). The value of the node represents the relative concentration to the control group of each irradiated subgroup after splitting, with a percentage of mice in each subgroup compared to the global population (40 mice). The value of the node represents the relative concentration to the control group of each irradiated subgroup after splitting, with a percentage of mice in each subgroup compared to the global population (40 mice). IL: interleukin; TNF: tumor necrosis factor.

(median level of the population) of the control level, with low levels below this threshold. Groups with low levels of naïve T-CD4+ cells exhibited significantly lower plasma levels of IL-6 and TNF- α than those with high levels of naïve T-CD4+ cells (Fig. 4E).

4.2.3. Decision tree regression for leukocyte subpopulations by cytokine levels following X-ray irradiation

Based on the results obtained from PCA, we aimed to investigate the associations between plasma levels of specific cytokines and their corresponding leukocyte subpopulations using a tree-based model following X-ray brain irradiation. The random forest model identified IL-6 and TNF- α as the two cytokines that most significantly influenced variations in neutrophils, monocytes, and naïve T-cells following brain irradiation (Fig. 5).

The decision tree model was subsequently employed to identify further the relationship between MCP-1, IL-6, TNF- α , IL-17a, and IFN- γ plasma levels and the counts of neutrophils, monocytes, and naïve T-cells following X-ray brain irradiation. The model revealed that a high TNF- α level (120% compared to that in control mice), correlates with an increase in naïve T-cells (of both CD4+ and CD8+), neutrophils, and monocytes (Fig. 5). Conversely, a low IL-6 level without a concurrent increase in TNF- α levels was linked to a decrease in naïve T CD4+ cells, neutrophils, and monocytes. In addition, reduced levels of IL-17A and IL-1 α without an increase in TNF- α were associated with a decrease in naïve T-CD8+ cells (Fig. 6).

5. Discussion

In this study, we not only analyzed changes in plasma cytokines following brain irradiation but also uncovered consistent association between cytokine profiles and leukocyte subpopulations through machine-learning techniques. Firstly, PCA provided a comprehensive overview, helping to identify key cytokines for further exploration within specific leukocyte subpopulations. Specifically, we found that changes in MCP-1, TNF- α , IL-6, IL-17A, and IFN- γ levels were associated with alterations in naïve T-cells, monocytes, and neutrophil counts after X-ray brain irradiation. The relationships between cytokine levels and leukocyte subpopulations following proton brain irradiation were more modest. Next, the random forest model indicated IL-6 and TNF- α as the two cytokines most significantly associated with changes in naïve T-cells, monocytes, and neutrophils post-X-ray brain irradiation. Finally, decision tree analysis specified these relationships, showing that high levels of TNF- α led to an increase in naïve T-cells, neutrophils, and monocytes, while low levels of IL-6 were linked to decreases in these cell counts.

Previous research has demonstrated that conventional X-ray irradiation in the thoracic or lung regions markedly enhances the expression of IL-6, IL-1 β , and TNF- α at the systemic level [5]. Besides, a study comparing the effects of X-ray and proton irradiation on the right hind leg indicated a significant reduction in the levels of IL-1 β , IL-6, IL-10, IFN- γ , and TNF- α with proton irradiation.

tion, relative to controls, five days postirradiation [19]. However, such changes were not detected in our investigations, possibly due to the differential tissue responses to irradiation. To the best of our knowledge, this study is the first to examine the expression of circulating cytokines in mice subjected to either X-ray or proton brain irradiation. Brain irradiation is known to potentially cause neuronal damage, triggering the release of pro-inflammatory cytokines at the tissue level [20]. Nonetheless, the alterations in circulating cytokine levels following brain irradiation have received limited attention. Blethen et al. reported a notable rise in the plasma levels of TNF- α immediately after a single whole-brain irradiation dose in immunocompetent mice. This increase, however, reverted to baseline within 12 h post-irradiation [21]. Consequently, the timing of sample collection is crucial for monitoring plasma cytokine level changes, as these alterations may occur immediately then recover following brain irradiation.

Our results revealed distinctive correlations between cytokine levels induced by X-rays and protons following fractionated brain irradiation in rodents. Following brain irradiation with X-rays, the variation in cytokine levels was highly linked to neutrophils, monocytes, and naïve T-cells. Previous rodent studies have indicated differences in the inflammatory response to proton and X-ray irradiation, with distinct regulation of principal pro-inflammatory cytokines [19]. The mechanisms underlying the effects of radiotherapy on normal tissues remain elusive, and predictive methods for such risks are currently lacking [22]. Radiation-induced abnormal cytokine production has been proposed as a contributing factor, supported by the significant positive correlations observed between b-FGF, IL-1 β , and TNF- α following radiotherapy in prostate cancer patients [22].

Our analysis showed a strong association between TNF- α and IL-6 levels and variations in neutrophils, monocytes, and naïve T-cells following X-ray brain irradiation. Radiation-induced activation of TNF- α expression has been documented since 1989, and clinical studies have suggested that its elevation in serum post-total-body irradiation may be linked to a higher risk of radiation-related complications [23,24]. IL-6 is known for its role in inducing acute-phase proteins and influencing leukocyte recruitment during inflammation [25,26]. It is particularly crucial in regulating neutrophil trafficking via the STAT3 signaling pathway and modulating monocyte activity towards a macrophage phenotype [27,28]. Beyond its influence on the innate immune response, IL-6 also enhances adaptive immune responses [26]. In addition, rodent studies have identified IL-6 as a critical cytokine for the homeostasis of naïve T-cells, independent of the conventional T-cell homeostatic cytokine, IL-7 [29]. Hence, within the context of our study on brain irradiation, the level of IL-6 could signify the maintenance of the T-cell population following their depletion due to brain irradiation. In addition, the observed variation in leukocyte subpopulations may contribute to cytokine variability following brain irradiation. During inflammation, TNF- α is released by various activated immune cells, including monocytes, macrophages, neutrophils, B cells, T-cells, and fibroblasts [30]. Consequently, the established positive correlation between TNF- α levels and neutrophils, monocytes, and naïve T-cells may reflect the activation of the immune system in response to brain irradiation.

6. Conclusion

In conclusion, our findings revealed distinct inflammatory responses triggered by X-ray irradiation compared with proton brain irradiation. This distinction is evidenced by the differential regulation of cytokines in the blood and their association with various leukocyte subpopulations. Variations in inflammation-regulating cytokines could potentially influence the efficacy of radiotherapy

and its integration with immunotherapy. However, further research is necessary to precisely elucidate how proton and X-ray irradiation affect the inflammatory response and radiotherapy effectiveness.

Disclosure of interests

The authors have not declared their activities/relationships/interests.

Funding

This project has received financial support from the CNRS through the CNRS 80|Prime program, Ligue contre le cancer and région Normandie (00129360-22E04690), programme PAUSE-ANR Ukraine (Fonds d'urgence UKRAINE–Collège de France et ANR-22-PAUK-0033-01/ANR-23-PAUK-9033-01).

Appendix A. Supplementary data

Supplementary data (cytokine analysis protocol) associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.canrad.2024.08.001>.

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