Universidade de Lisboa
Faculdade de Motricidade Humana

Effect of exercise on immune system markers in cancer patients and survivors: a systematic review

Dissertação elaborada com vista à obtenção do Grau de Mestre em

Exercício e Saúde

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2023

Acknowledgments/Agradecimentos

Bem…parece que este capítulo está a acabar e que estou a terminar mais uma etapa nesta longa caminhada. Foi sem dúvida uma experiência bastante enriquecedora e na qual aprendi bastante, mas, acima de tudo, quero agradecer a todas as pessoas que fizeram parte e me apoiaram ao longo deste percurso.

Em primeiro lugar, gostaria de agradecer ao Professor Doutor Luís Bettencourt Sardinha pela oportunidade de realizar esta dissertação. Foi seguramente um desafio e uma grande oportunidade de aprendizagem, a qualidade e o rigor incutido exigiram que fizesse e quisesse ser melhor todos os dias. Agradeço-lhe a oportunidade.

Inês e João, para vocês nem tenho palavras. Agradeço-vos, do fundo do coração, pela incansável ajuda que me deram ao longo de todos estes anos. Desde o primeiro dia que me acolheram no laboratório e se disponibilizaram para me ajudar em tudo o que precisasse. São claramente uma parte essencial deste trabalho, por todo o apoio e motivação que me deram e dão. Inês, desde o início desta dissertação que estiveste lá para me ajudar, todas as palavras e dicas que me deste e dás, a partilha de conhecimento, a disponibilidade e apoio, a exigência e rigor que impões. Não teria sido possível sem ti, foste e és incansável e, ainda que, um obrigada não seja suficiente para te agradecer por tudo, obrigada Inês! João, agradeço-te pela confiança que depositas em mim, pelas *pep talks*, pela exigência, pelas críticas e pelos conselhos que me dás, pela preocupação e pela motivação, obrigada por tudo!

No entanto, não posso deixar de agradecer às restantes pessoas que me acompanharam e acolheram no Laboratório de Exercício e Saúde, todos os dias ao longo deste tempo. Ao Gil e ao Tomás, os meus queridos colegas de gabinete e euro milhões, ainda havemos de ganhar o jackpot! Ao Filipe, à Catarina, ao Ruben e à Megan, obrigada por todos os almoços, pelo convívio, pelo apoio e motivação. Sem dúvida que seria bem mais complicado completar este processo sem todos vocês.

Agradeço também à afilhada que mais me atura e ouve os meus dramas e ansiedades, à companhia de casa à faculdade e da faculdade a casa, seja cedinho ou bem tarde, aos almoços, lanches e jantares todos, pela companhia no gabinete, por estares lá para ouvir as lamúrias e para acompanhares as conquistas. Espero que já saibas quantos pedais há num carro e quantas turmas de 2º ano havia ahah, não podia não gozar contigo nestes agradecimentos Ana! Aproveito e agradeço também à Costinha, pela companhia, pelo apoio e pelos momentos todos deste ano. Desde o teu primeiro ano, que sabia que ias ser boa colega (e amiga) ahah. Que o nosso trio se vá mantendo e que as fofocas nunca acabem!

E a todos os amigos e colegas que fizeram parte deste percurso, agradeço a companhia e a partilha de tantos momentos. Mas, em especial, à minha melhor amiga, a que está sempre lá há tantos anos, nos bons e nos maus momentos, a que nunca me deixa, a que me apoia sempre e a que tem sempre as melhores palavras para mim. Um obrigada já não chega, Tita! À Bea, não há forma de te agradecer por tudo mas agradeço-te por todo o apoio e força que me dás diariamente, por estares lá sempre para mim, não há palavras para ti, o resto tu sabes. Clu, não me poderia esquecer de ti, obrigada por seres a minha companhia de Marvel e que façamos muitas mais viagens juntas! Às restantes girls, Manso, Gomes e Luz, obrigada por todos os jantares, viagens, convívios, obrigada por tudo! A ti Matos, obrigada por seres o mais parecido que tenho de parceiro da tese, já são muitos (muitos mesmo) anos na mesma turma e em percursos parecidos, obrigada pela companhia, pelos cafés e pelas centenas de boleias até à faculdade.

Por fim, agradecer à minha família, por estarem sempre lá para me apoiar e ajudar em tudo o que possa precisar. À minha mãe, por toda a motivação, força, carinho, apoio e amor que me deu sempre. És e serás para sempre a minha inspiração. Espero que estejas orgulhosa de mim. Ao meu pai e aos meus avós, obrigada pelo apoio, pela força e pela ajuda que me deram e dão todos os dias. À tia Lena, pelos momentos e

palavras que dão mais um bocadinho de força para trabalhar e pelo apoio. E a tantos outros, obrigada por acompanharem este meu percurso. Obrigada!

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Abbreviations

1RM	1 Repetition maximum
BC	Breast cancer
BMD	Bone mineral density
BMI	Body mass index
CRF	Cardiorespiratory fitness
CTx	Chemotherapy
DNA	Deoxyribonucleic acid
FM	Fat mass
HIIT	High-intensity interval training
MICT	Moderate intensity continuous training
MVPA	Moderate-to-vigorous physical activity
NCR	Natural cytotoxicity receptor
NK cells	Natural killer cells
NKCA	Natural killer cytotoxic activity
NSCLC	Non-small cell lung cancer
PA	Physical activity
PBMCs	Peripheral blood mononuclear cells
PEDro	Physiotherapy Evidence Database
RCTs	Randomized controlled trials
RPE	Rate of perceived exertion
T1/T2	IFN-γ-producing CD3+ T lymphocyte/IL-4-producing CD3+ T lymphocytes
Tc1/Tc2	CD3+ T lymphocyte subset - cytotoxic T cell type 1/ CD3+ T lymphocyte subset - cytotoxic T cell type 2
TCR	T-cell receptor

TH1/TH2	CD3+ T lymphocyte subset - helper cell type 1/ CD3+ T lymphocyte subset helper cell type 2
Treg	Regulatory T cells
VO_{2peak}	Peak oxygen uptake
WHO	World health organization

Abstract

Purpose: To present a systematic review on randomized controlled trials that analyzed the impact of aerobic, resistance, combined, and Tai Chi exercise on the immune system in cancer patients and survivors.

Methods: Pubmed, Web of Science, the Cochrane Library, and Scopus databases were searched to identify pertinent RCTs. Randomized controlled investigations that looked at the effect of exercise interventions with immune system markers as one of the outcomes were included. The methodological scale to evaluate the included investigations was the PEDro scale (Physiotherapy Evidence Database).

Results: Eleven articles met all the inclusion criteria. PEDro scale score ranged between 5 and 10 points, with an overall average of 6.9 points, with 2 investigations of excellent quality, 7 of good quality, and 2 of low quality. Of the 11 investigations, 3 observed beneficial results on the effect of aerobic, resistance, combined, and Tai Chi exercise on immune cells (NK cells) in cancer patients and survivors, and 8 reported no effects.

Conclusion: Few investigations suggest a between-group effect of exercise on the immune system in cancer patients and survivors when compared to a control group. More high-quality studies are needed in different phases and stages of treatment and disease and in different types, intensities, and durations of physical exercise.

Keywords: Aerobic exercise; resistance exercise; combined exercise; Tai Chi

Resumo

Objetivo: Apresentar uma revisão sistemática de investigações randomizadas controladas que analisaram o impacto de exercício aeróbio, de resistência, combinado e Tai Chi no sistema imunitário de pacientes com cancro e sobreviventes.

Métodos: As bases de dados da Pubmed, Web of Science, the Cochrane Library e Scopus foram usadas para identificar RCTs pertinentes. As investigações randomizadas controladas que analisavam o efeito das intervenções de exercício com os marcadores do sistema imunitário como um dos *outcomes* foram incluídos. A escala metodológica para avaliar as investigações incluídas foi a escala PEDro (Physiotherapy Evidence Database).

Resultados: Onze artigos preencheram todos os critérios de inclusão. A pontuação da escala PEDro variou entre 5 e 10 pontos, com uma média geral de 6.9 pontos, sendo 2 investigações de excelente qualidade, 7 de boa qualidade e 2 de baixa qualidade. Dos 11 investigações, 3 observaram resultados benéficos sobre o efeito do exercício aeróbio, de resistência, combinado e de Tai Chi sobre as células imunitárias (células NK) em pacientes e sobreviventes com cancro e 8 não relataram efeitos.

Conclusão: Poucas investigações sugerem um efeito intergrupo do exercício no sistema imunitário, em pacientes com cancro e sobreviventes, comparativamente com o grupo de controlo. São necessários mais estudos de alta qualidade nas diversas fases e estadios de tratamento e da doença e em diferentes tipos, intensidades e durações de exercício físico.

Palavras-chave: Exercício aeróbico; exercício de resistência; exercício combinado; Tai Chi

Chapter 1

Theoretical Framework

Thesis overview

The worldwide cancer incidence rate has been increasing and is expected to continue to increase in the next years. In 2020, there were an estimated 19.3 million new cases worldwide, with a 47% increase in prevalence values by 2040 (Sung et al., 2021). In Portugal, there were approximately 60 467 new cases in 2020, in which colorectal, breast, prostate, and lung cancer were the more incident cancers (WHO, 2021a).

Exercise in cancer patients is associated with beneficial alterations in objective physiological measures (e.g., aerobic and muscular fitness, muscular strength and endurance) and patient-reported results (e.g., quality of life, depression, and cancer-related fatigue (Christensen et al., 2018)) (Segal et al., 2017). Moreover, emerging evidence suggests that exercise is directly linked to tumor biology control, and hence, with the potential to improve the clinical outcome (Hojman et al., 2017).

The immune system's capacity to detect, identify and eliminate infectious pathogens such as viruses, bacteria, and fungi is an important and powerful intrinsic weapon against cancer (Weinberg, 2014). Due to immune system surveillance, multiple virus-induced cancers can be prevented from developing (Hanahan & Weinberg, 2011). The immunological profile of tumors is closely related to the prognosis of cancer and, therefore, a better prognosis is associated with high levels of infiltrating natural killer (NK) cells and cytotoxic T cells in tumors of cancer patients (Hojman et al., 2017). Exercise has been shown to regulate the cellular immune system since cytotoxic immune cells are mobilized to the circulation during exercise through different mechanisms that involve adrenergic signalling and blood flow-induced shear stress (Christensen et al., 2018). Thus, even though the mechanisms through which exercise can improve the immune system of cancer patients have been outlined, more intervention studies are warranted in this research field to address its efficacy in the human model and on different types of cancer.

This thesis is divided into two chapters. The first chapter includes 1) a review of the existent literature that comprehends the epidemiology and physiopathology of cancer; 2) the immune system's organization and its relationship with cancer disease; 3) a description of the effects of PA on cancer patient's immune system; 4) a description of the effects of aerobic, resistance, combined exercise and Tai Chi on cancer patient's immune system and the mechanisms linking exercise and the alterations in the immune system. The second chapter consists of a systematic review (i.e., full paper) of randomized controlled trials (RCTs) describing the effects of exercise on the immune profile in cancer patients.

Literature Review

1. Cancer

1.1. Epidemiology of Cancer

According to the World Health Organization (WHO), cancer is the leading cause of death worldwide, with nearly 10 million deaths in 2020 (WHO, 2022). In 2020, there were an estimated 19.3 million new cases of which men accounted for 10.1 million and women for 9.2 million (Sung et al., 2021). The three most common cancers worldwide were breast, lung, and colorectal, contributing 11.7%, 11.4,% and 10%, respectively of the total number of new cases diagnosed in 2020 (Sung et al., 2021). The most common cancer in men was lung cancer (14.3%), while in women was breast cancer (BC) (24.5%) (WHO, 2021b)

In Portugal, in 2020, there was a total of 60 467 new cases of cancer and 30 168 deaths (WHO, 2021a). Of these, men accounted for 33 784 cases, whereas women accounted for 26 673 cases (WHO, 2021a). The three most common cancers in Portugal were colorectal, breast, and prostate cancer, contributing 17.4%, 11.6%, and 11.2% of the total incidence, respectively (WHO, 2021a). The cancer with the highest incidence in women was in the breast region (26.4%) and in men it was the prostate (20%) (WHO, 2021a).

1.2. Physiopathology

Cancer cells derive from normal cells that are no longer able to assemble and develop tissues with a normal structure and function (Weinberg, 2014). According to their level of growth and aggressiveness, tumors can be classified into: benign (those that spread only locally without invading the adjacent tissues) and malignant (those that invade nearby tissues and spawn metastases) (Weinberg, 2014). Tumor development is a complex multi-step process (Lambert et al., 2017), involving several modifications (activation of oncogenes and repression of tumor suppressor genes) on cells and on

their physiologic regulatory systems, which typically takes decades to complete (Weinberg, 2014). Tumor progression is the process by which normal cells transform into cells that exhibit progressively more neoplastic traits (Patel et al., 2019). The formation of a tumor is caused by a sequence of randomly occurring mutations and epigenetic changes in DNA that affect the genes that control cell proliferation, survival, and other characteristics linked to the malignant cell phenotype (Lambert et al., 2017).

Throughout the tumor's multi-step progression and growth, they develop several biological traits that are considered to be the hallmarks of cancer (Hanahan & Weinberg, 2011). These hallmarks (figure 1) serve as a guiding principle for organizing the complexity of neoplastic disease (Hanahan & Weinberg, 2011). In 2000, the first six hallmarks were described and over the years, through conceptual advancement, more hallmarks have been characterized. Currently, there are fourteen hallmarks of cancer described (Hanahan, 2022; Hanahan & Weinberg, 2011).

Figure 1 - Hallmarks of Cancer (adapted from (Hanahan, 2022), page 43)

- **Sustaining proliferative signalling** – cancer cells have the ability to stimulate their growth, and as opposed to normal cells, they do not depend on external signals.
- **Evading growth suppressors** – cancer cells are capable of resisting inhibitory signals that would prevent them from growing, through the inactivation of tumor suppressors.
- **Nonmutational epigenetic reprogramming** – purely epigenetically regulated changes in gene expression occur as part of an independent mode of genome reprogramming.
- **Avoiding immune destruction** - certain cancer cells can develop defenses to avoid detection and elimination by the host's immune system.
- **Enabling replicative immortality** – unlike most normal cells that are only able to undergo a limited number of subsequent cell growth and division cycles, the replicative capacity of cancer cells is limitless.
- **Tumor-promoting inflammation** – cancer cells have the capacity to manipulate inflammatory mechanisms to aid in their survival and growth.
- **Polymorphic microbiomes** – the polymorphic variability in the microbiomes can significantly affect the phenotypes of cancer, which means that some types of bacteria and fungi can have either beneficial or detrimental consequences on the progression, malignant development, and response to treatment of cancer.
- **Activating invasion and metastasis** – tumor cells appeal to mechanisms that allow them to expand into other environments and tissues, invade other tissues, and form small nodules of cancer cells that then grow into macroscopic tumors.
- **Inducing or accessing vasculature** – tumors need nutrients, so cancer cells can induce angiogenesis.
- **Senescent cells** – senescent cells in various ways promote tumor growth and the spread of cancer, since they contribute to proliferative signalling, avoid apoptosis,

suppress tumor immunity, promote invasion and metastasis, and induce angiogenesis.

- **Genome instability and mutation** – tumor cells have a higher sensitivity to mutagenic agents, which contribute to the damage of multiple genes that regulate cell division and tumor suppression.
- **Resisting cell death** – cancer cells present a variety of mechanisms to prevent or delay apoptosis.
- **Deregulating cellular metabolism** - cancer cells present abnormal metabolic pathways since they need a lot of energy to stimulate cell growth and division, so they utilize “aerobic glycolysis”.
- **Unlocking phenotypic plasticity** - cancer cells unlock phenotypic plasticity to evade or escape from the terminal differentiation state, which means that they can reverse their state and enable different types of disrupted differentiation.

2. Immune System

2.1. Immune System’s organization

The immune system has the function to protect our body by recognizing, attacking, and destroying elements that are foreign to our organism (Gleeson, 2006). The immune system can be divided into two components – innate and adaptive (McComb et al., 2019).

The **innate** component, also known as natural or nonspecific immunity, consists of **cellular** elements (monocytes, macrophages, neutrophils, dendritic cells, mast cells, basophils, eosinophils, and innate lymphoid cells (with three subsets that include ILC1, NK cells, ILC2, the lymphoid tissue inducer cells, the natural cytotoxicity receptor (NCR)-ILC3s, and NCR+ ILC3 cells) (Krause, 2019), soluble elements (acute-phase proteins, complement, lysozymes, cytokines - interleukins, interferons, colony-stimulating factors, and tumor necrosis factors), physical barriers (such as the skin and mucous membranes – gut and respiratory tract), and the reticuloendothelial system (Gleeson, 2006). The

innate immune system is the first line of defense against invasive microorganisms, intending to restrict their entry into the body by providing a) physical/structural obstruction and clearance processes using mucus, ciliary function, skin, and mucosal barrier's epithelial linings, and peristalsis; b) chemical elements, such as the abundance of antimicrobial peptides and proteins and the low pH of stomach fluids; c) phagocytic cells, such as eosinophils, neutrophils, blood monocytes, tissue macrophages and dendritic cells that can absorb and destroy germs; d) NK cells, which are generalized killer cells that may eliminate virally-infected host cells and stop further viral replication (Gleeson, 2006). When the innate system is compromised, it usually results in the activation of the adaptive immune system, which contributes significantly to the recovery of the infection (Krause, 2019).

The **adaptive** component, also known as acquired or specific immunity, includes cellular (T-cells and B-cells) (Krause, 2019) and soluble components (immunoglobulins (IgA, IgD, IgE, IgG, IgM)) (Nicholson, 2016). The T lymphocytes cells most known are CD4+ and CD8+; CD4+ T cells are known as helper cells, since they cooperate with other immune cells in their function, while CD8+ T cells are known as cytotoxic T cells and are responsible for recognizing tumor cells and virus or bacteria-infected cells and destroying them (Krause, 2019). B cells divide into two categories – B1 and B2 cells -, while B1 cells are regarded as innate-like cells and B2 cells are associated with the adaptive immune system, both express an immunoglobulin receptor (Krause, 2019). Immunoglobulins, also known as antibody receptors, are antigen-specific receptors, present several isotypes (IgD, IgM, IgG, IgE, and IgA), and are essential to neutralize infections before they start (Nicholson, 2016). Antibodies bind to specific antigens (Megha & Mohanan, 2021), and, at the same time, signal to immune cells (Nicholson, 2016), becoming essential in the immune response, as they have the ability to destroy a wide range of microorganisms and antigens, avoiding destructive mechanism against the host's tissue (Megha & Mohanan, 2021). The main goal of acquired immunity is to

counteract infections by avoiding pathogen colonization and keeping them out of the body (immune exclusion), as well as to locate and eradicate particular invading germs (immune elimination) (Gleeson, 2006).

2.2. Cancer and Immune System

In the periphery of tumor-burdened hosts, there have been observed perturbations in dendritic cells, which are crucial orchestrators of CD8+ and CD4+ T cell priming, differentiation, and proliferation in many contexts, including cancer, and this has important effects on the development of antitumor immune responses, (Hiam-Galvez et al., 2021). When compared to healthy control donors, the number of dendritic cell subsets is reduced in the peripheral blood of people with several different types of cancer (Hiam-Galvez et al., 2021).

Other immune cells are also functionally perturbed, such as peripheral T cells, given that polyclonal memory CD4+ and CD8+ T cells from peripheral blood exhibit abnormalities in the production of interferon-gamma (INF-y) and IL-2 – there is a decrease in capacity to produce IL-2 and IFN-y, especially in BC patients (Hiam-Galvez et al., 2021). However, the production of TNF-α remains unaffected in patients with primary and metastatic breast, lung, or melanoma cancer (Verronese et al., 2016). The causes of these deliberate modifications are yet unknown (Verronese et al., 2016). The most researched T cell alteration in cancer is the increase of suppressive CD4+ regulatory T cells (Treg) in the periphery and their infiltration into the tumor (Hiam-Galvez et al., 2021). Recent research has demonstrated that intratumoral suppressive Treg cells are derived from naturally occurring thymic Treg cells instead of from tumor-induced differentiation of naive CD4+ T cells since intratumoral suppressive Treg cells share phenotypic and T-cell receptor (TCR) repertoires with intratumoral T cells in the blood of cancer patients (Hiam-Galvez et al., 2021). B cells, which are distinguished by the production of the anti-inflammatory cytokine IL-10, are another suppressive lymphocyte population that participates in the development of tumors (Hiam-Galvez et al., 2021).

Similar to Treg cells, regulatory B cells that produce the cytokine IL-10 have been shown to increase in the peripheral blood of patients with lung and gastric cancer while total B cell frequencies remained stable (Hiam-Galvez et al., 2021).

Another crucial element of antitumor immunity is NK cells, which have the ability to both directly destroy tumor cells and modify the antitumorigenic behavior of other immune cells (Hiam-Galvez et al., 2021). Cancer patients whose tumors have higher levels of infiltrating NK cells and cytotoxic T cells are associated with a better prognosis (Hojman et al., 2017).

3. Physical Activity, Immune System, and Cancer

Physical activity (PA) is described as any body movement produced by the skeletal muscle in which the energy expenditure is raised above the resting values (Caspersen et al., 1985). On the other hand, exercise is a subset of PA that is planned, structured, repetitive, and has a specific goal to improve a given trait of physical fitness (Caspersen et al., 1985). Regular PA of moderate intensity or higher is linked to a reduced risk of different types of cancer, such as breast, colon, and endometrium cancer (McTiernan, 2008). Indeed, this observational evidence has been replicated in other investigations, with an association between regular moderate-vigorous PA (MVPA) and a lower risk for developing several cancers being found, such that proximal (-24%) and distal (-23%) colon, endometrial (-17%), breast (-12%), prostate (-10%), gastroesophageal (-18%), ovarian (-11%), renal (-12%), lung (-24%) and pancreatic (-11%) (Ruiz-Casado et al., 2017). On a more specific domain, leisure time in MVPA (i.e. walking, running, or swimming), has also been shown in several prospective cohorts, to be associated with a significantly reduced risk for ten cancers (Ruiz-Casado et al., 2017). Several associations like the ACSM and the American Cancer Society implemented PA recommendations for cancer survivors, that established that cancer survivors should return to normal daily activities as soon as possible and suggest that adults should engage in 150 to 300 min/per week of moderate-intensity activity or 75-150 min/week of

vigorous-intensity activity (or a correspondent combination) (Rock et al., 2022; Wolin et al., 2012) and muscle-strengthening activities at least on 2 days a week (Rock et al., 2022). The mechanisms behind the effects of PA and exercise will be addressed in the next subchapter.

4. Exercise, Immune System, and Cancer

4.1. Cancer and Aerobic Exercise

Literature has shown that aerobic exercise in cancer patients has adaptive responses (whole-body adaptations, muscle adaptations, systemic adaptations, and health-related quality of life) (Hojman et al., 2017). Considering whole-body adaptations, the literature suggests that aerobic exercise improves cardiorespiratory fitness (CRF) and peak oxygen uptake (VO_{2peak}), but has selective effects on body composition (i.e., no changes in body mass index (BMI), fat mass (FM), and bone mineral density (BMD)) (Sasso et al., 2015); moreover, aerobic training promotes noticeable improvements in several metabolic outcomes such as those related with insulin sensitivity (e.g. insulin levels, and leptin) and inflammatory markers (e.g. C-reactive protein) (Ballard-Barbash et al., 2012) (Hojman et al., 2017)

Evidence suggests that aerobic exercise can increase the levels of several markers related to the immune system (figure 2), such as leukocytes, lymphocytes, granulocytes, neutrophils, eosinophils, and monocytes, in healthy individuals (Goncalves et al., 2019). Relatively to CD4+ and CD8+, there are some studies in healthy subjects, with both acute or chronic exercise, that indicate that aerobic exercise can increase the two of them (A. LaPerriere & Fletche, 1994; Gannon et al., 2001; Moyna, 1996), and other studies that only reported increases in CD8+ (Kurokawa 1995; Nehlsen-Cannarella, 1991). Increases in NK cells after aerobic exercise are also reported in some studies (Gannon et al., 2001)

4.2. Cancer and Resistance Exercise

Resistance exercise also has adaptive responses (whole-body adaptations, muscle adaptations, systemic adaptations, and health-related quality of life) in cancer patients (Hojman et al., 2017). When looking at whole-body adaptations, the literature suggests that resistance exercise slightly increases CRF, body weight, and lean mass, but has no significant effects on FM and percentage of body fat (Courneya et al., 2007); considering muscle adaptations, significant increases in muscle strength (1RM) and muscle mass had been reported, while no effects on muscle fiber size and type composition and capillary density were observed (Courneya et al., 2007; McNeely et al., 2008); as far as systemic adaptations, no assessments were made (Ballard-Barbash et al., 2012) (Hojman et al., 2017)

A recent pilot on the effects of resistance exercise in ovarian cancer survivors suggested that this type of intervention increases markers related to the immune system - Th1, Th2, Th1/Th2 ratio, CD4+, CD8+ (figure 2), while also increasing muscle strength and endurance, when compared to the control group (Lee & Jee, 2021).

4.3. Cancer and Combined Exercise

Literature on the effects of combined exercise on the immune system of cancer patients and cancer survivors is scarce. However, when looking at other health conditions, combined aerobic and resistance exercise in men with obesity showed no significant differences in leucocytes and NK cells levels (Jin et al., 2018); combined exercise in HIV/AIDS demonstrated significant increases in TCD4+, but not in TCD8+ and TCD4+/TCD8+ (Garcia et al., 2014).

Combined training has shown that, in BC survivors, there is an improvement in VO_{2peak} and muscle strength while there are no significant changes in measures related to body composition – BMI, waist circumference, fat percent, and lean body mass, and related to systemic metabolic adaptations – insulin, leptin, and glucose levels (Dethlefsen et al., 2016). In men with prostate cancer undergoing androgen suppression therapy, the

intervention group demonstrated improvements in lean mass (total body, upper and lower limb, and appendicular skeletal muscle) and muscle strength measured through 1RM (chest press, seated row, leg press, and leg extension), while in systemic adaptations – insulin and glucose levels – there are no significant alterations (Galvao et al., 2010).

4.4. Cancer and Tai Chi

Literature on the effects of Tai Chi on the immune system has demonstrated that this type of exercise has small but significant effects on innate immune cells (dendritic cells, eosinophils, monocytes, and neutrophils), showing an increase in their levels when compared to the control groups, and also has a small effect on increasing the quantity of adaptive immune cells when compared with the control group (Oh et al., 2020). The evidence on NK cells is still not clear, with some studies showing significant decreases (Oh et al., 2020) and others made on middle-aged healthy women reporting no significant effects (Liu, 2012).

Evidence on post-treatment BC survivors demonstrated that NKG2D and white blood cells increased significantly in the intervention group between the baseline and the end of the intervention at 52 weeks (Cheung, 2021). In addition, evidence on subjects with non-small lung cancer survivors suggests that Tai Chi may significantly improve the percentage of CD4, CD8, and the CD4:CD8 ratio within-group before and after the exercise intervention but does not report significant differences between the Tai Chi group and the control group (Zhang et al., 2013).

4.5. Exercise and Cancer Hallmarks

As detailed previously, there are fourteen cancer hallmarks described, and Ruiz-Casado elegantly reviewed the impact of exercise on 9 out of the 14 hallmarks, which will be explored in this chapter (Ruiz-Casado et al., 2017):

- **Sustaining proliferative signalling** – exercise induces decreases in circulating levels of multiple hormones that affect cell proliferation and that avoid apoptosis.
- **Evading growth suppressors** – exercise may have a positive impact on growth suppressors, such as p53 and retinoblastoma protein.
- **Avoiding immune destruction** - exercise increases the immunological response and can positively impact human immunity, through the increase in lymphocyte proliferation, NK cytotoxic activity, cytolytic capacity of macrophages, alveolar macrophage antitumor cytotoxicity, and may reduce the immunosuppressive effects of Tregs.
- **Enabling replicative immortality** – exercise has a positive impact on telomeres length, hence an active lifestyle may have a protective effect and delay biological aging.
- **Tumor-promoting inflammation** – it appears that exercise can decrease the infiltration of macrophages and the accumulation of neutrophils in the tumor tissues.
- **Activating invasion and metastasis** – even though there is no consensus on the impact of exercise on metastasis formation, exercise may increase cadherins and may modulate the blood-brain barrier integrity.
- **Inducing or accessing vasculature** – exercise promotes intratumoral perfusion and vascularization, thus, possibly improving drug delivery.
- **Resisting cell death** – studies in the animal model showed that exercise suppresses tumor growth, stimulates apoptosis, and increases caspase-3 levels.
- **Deregulating cellular metabolism** - data on the impact of exercise on this hallmark is scarce, however, it appears that exercise can affect mechanisms that regulate glucose homeostasis and reduce levels of proteins that participate in cell proliferation.

Evidence on the most recent hallmarks, such as nonmutational epigenetic reprogramming, polymorphic microbiomes, senescent cells, genome instability and mutation, and unlocking phenotypic plasticity, has still not been detailed (Ruiz-Casado et al., 2017).

4.6. Exercise, Immune System, and Mechanisms

Exercise has been shown to regulate the cellular immune system since cytotoxic immune cells are mobilized to the circulation during exercise through different mechanisms that involve adrenergic signaling and blood flow-induced shear stress (Idorn & Hojman, 2016). Moreover, both short-term and long-term exercise modify the functionality and number of immune cell subsets that make up the innate and adaptive immune systems in the circulation as well as in specific tissue compartments (Koelwyn et al., 2017). Through reprogramming or altered myeloid cell abundance in the tumor microenvironment, exercise may control innate immunity in cancer (Koelwyn et al., 2017). Exercise might reduce the number of neutrophils and macrophages that build up in the tumor microenvironment and it can also affect the number and activity of NK cells (Koelwyn et al., 2017). However, regarding the effects of exercise on adaptive immunity, little is known (Koelwyn et al., 2017). Following an exercise session/intervention, the body experiences both acute and long-term alterations in a variety of biological, epigenetic, metabolic, and inflammatory processes (Thomas et al., 2017). Nevertheless, it is not yet known which of these either alone or in combination has the most impact on the pathways that lead to cancer (Thomas et al., 2017). There are several potential mechanisms, nevertheless, this thesis will focus on immunity-related mechanisms.

The total number and composition of circulating leukocytes are significantly affected by a single exercise session (Simpson et al., 2015). Higher amounts of neutrophils, lymphocytes, and monocytes, including NK cells, CD4+ T cells, and B cells, are produced during exercise as a result of elevated catecholamine levels, which may also improve immune surveillance against cancer (Thomas et al., 2017). Of all the lymphocytes, NK

cells have the highest density of β-adrenergic receptors, which makes these cells highly responsive to the catecholamine's concentration (Idorn & Hojman, 2016). The NK cells' exercise-mediated mobilization is very fast, making NK cells the most responsive immune cell, taking as little as 70 seconds of exercise to increase the frequency of these cells in the blood by 6-fold (Idorn & Hojman, 2016). Conversely, if the exercise is excessively demanding, it is followed by lower lymphocyte concentrations and poor cellular-mediated immunity (Thomas et al., 2017). However, when following moderate exercise interventions, the majority of long-term research indicates that exercise increases immune function across all ages, especially with regular training (Thomas et al., 2017).

Exercise also has an impact on blood perfusion, body temperature, and oxygen consumption, which may also affect NK cell function (Idorn & Hojman, 2016). Studies have shown that exercise is associated with increased angiogenesis and vascularization (McCullough et al., 2014), which is linked with better blood perfusion (Garcia et al., 2016) and reduced tumor growth since the accessibility of circulating immune cells and medication to tumors is increased and there is less intratumoral hypoxia (McCullough et al., 2013; McCullough et al., 2014). Evidence shows that hypoxia inhibits NK cell-mediated lysis, possibly decreasing the cytotoxicity of tumor-infiltrating NK cells (Idorn & Hojman, 2016). Another mechanism that can also increase immune cell influx and function is hyperthermia (Hojman et al., 2017). Hyperthermia controls and delays tumor growth by increasing intratumoral NK cell infiltration; it also improves the circulation of immune cells by increasing the diameter of intratumoral blood vessels and by modifying the tumor vasculature by inducing IL-6 trans-signaling, allowing cytotoxic T-cell trafficking into tumors (Hojman et al., 2017). The overall effects of exercise on the immune system were summarized in figure 2.

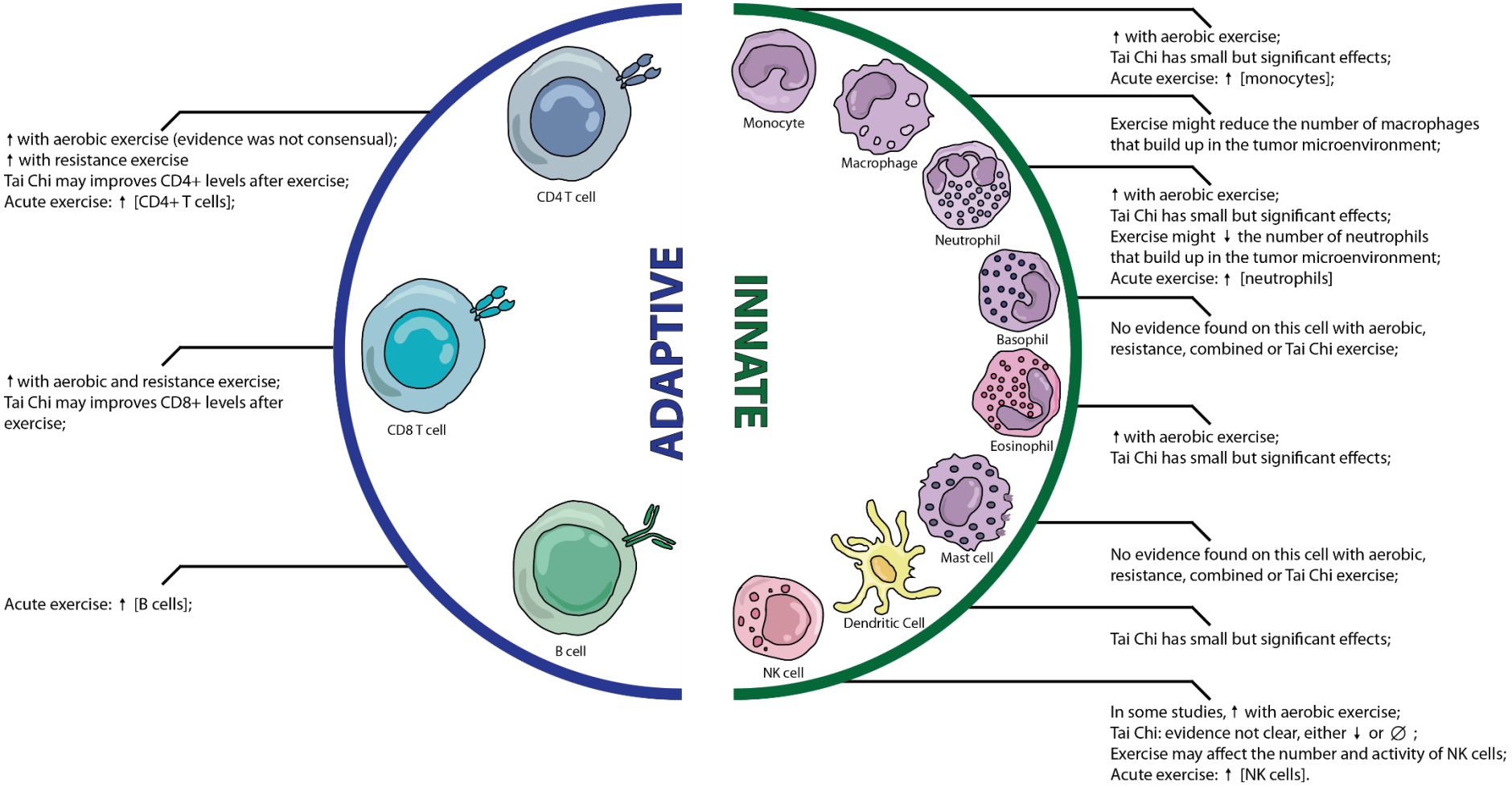

Figure 2 - Evidence of the effect of aerobic, resistance, combined, and Tai Chi exercise on the adaptative and innate immune cells.

Thesis's purpose

Even though the literature on cancer and exercise oncology has been increasing over the last few years, the evidence of different types of exercise, such as aerobic, resistance, combined, and Tai Chi, on the immune system is still not clear. To the best of our knowledge, there is still not been conducted any systematic review on this theme. Therefore, this systematic review aims to gather randomized controlled trials (RCT) studies that assessed the impact of aerobic, resistance, combined, and Tai Chi exercise on alterations in outcomes related to the innate immune system (NK cells and phagocytes) and adaptive immune system (T-cells and B-cells) in cancer patients and survivors. We hypothesized that the immune system-related outcomes would be improved with the practice of exercise.

Chapter 2

Systematic Review

Effect of exercise on immune system markers in cancer patients and survivors: a systematic review

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Abstract

Purpose: To present a systematic review on randomized controlled trials that analyzed the impact of aerobic, resistance, combined, and Tai Chi exercise on the immune system in cancer patients and survivors.

Methods: Pubmed, Web of Science, the Cochrane Library, and Scopus databases were searched to identify pertinent RCTs. Randomized controlled investigations that looked at the effect of exercise interventions with immune system markers as one of the outcomes were included. The methodological scale to evaluate the included investigations was the PEDro scale (Physiotherapy Evidence Database).

Results: Eleven articles met all the inclusion criteria. The PEDro scale score ranged between 5 and 10 points, with an overall average of 6.9 points, with 2 investigations of excellent quality, 7 of good quality, and 2 of low quality. Of the 11 investigations,3 observed beneficial results on the effect of aerobic, resistance, combined, and Tai Chi exercise on innate (NK cells) and adaptive cells in cancer patients and survivors, and 8 reported no effects.

Conclusion: Few investigations suggest a between-group effect of exercise on the immune system in cancer patients and survivors when compared to a control group. More high-quality studies are needed in different phases and stages of treatment and disease and in different types, intensities, and durations of physical exercise.

Keywords: Aerobic exercise; resistance exercise; combined exercise; Tai Chi

1. Introduction

According to the International Agency for Research on Cancer, the burden of cancer death and incidence is quickly rising worldwide (Sung et al., 2021). In 2020, about 19.3 million new instances of cancer were detected worldwide, with BC, lung cancer, and colorectal cancer accounting for the majority of new cases (WHO, 2021b). Globally, it is expected an increase of 47% in 2040, representing a total of 28.4 million new instances of cancer (Sung et al., 2021).

It is widely recognized that cancer patients can benefit from exercise training since it reduces cancer development and growth (Hojman et al., 2017) through tumor intrinsic (i.e. tumor progression) and extrinsic (i.e., improves physical fitness) effects (Idorn & Hojman, 2016). Further, also immune system adaptations derived from exercise practice play an important role in tackling tumor cell proliferation. Indeed, exercise stimulates blood perfusion and angiogenesis, oxygen consumption, and body temperature, which affect immune cell function, with a special emphasis on NK cells (Idorn & Hojman, 2016). Moreover, exercise, both in the short-term and long-term, affects the number and functionality of several immune cell types that make up the innate and adaptive immune system in the circulation and in specific tissue compartments (Koelwyn et al., 2017). Even though the majority of the available evidence focuses on the animal model, there is also research on humans that demonstrates that exercise regulates the cellular immune system, by helping the suppression of tumor growth and the immune cell mobilization (Hojman et al., 2017), through an increase in NK-cell infiltration (Djurhuus et al., 2023), an acute and gradual increase in the frequency of monocytes, lymphocytes, and neutrophils (Christensen et al., 2018) and an increase in the recruitment of leucocytes into the peripheral blood (Thomas et al., 2017). Cancer patients that engage in exercise are more likely to complete the planned dosage of treatment (Christensen et al., 2018)

To date, there is a limited number of studies performed on this topic, with evidence on the different types of exercise showing that aerobic exercise has benefits on several immune cells and increases T and NK cells; resistance exercise appears to also increase CD4+ and CD8+ and Th1, Th2 and Th1/Th2 ratio; no evidence on combined exercise was found and that Tai Chi has small but significant effects on innate and adaptive immune cells. Hence, a systematic review may help to understand what is published and known about the effect of aerobic, resistance, combined, and Tai Chi exercise on the immune system of cancer patients and if future research is needed in this area. Therefore, the purpose of this investigation was to perform a systematic review of randomized controlled trials (RCTs) that assessed the impact of aerobic, resistance, combined, and Tai Chi exercise on alterations in outcomes related to the innate immune system (NK cells and phagocytes) and adaptive immune system (T-cells and B-cells).

2. Methodology

Searches were conducted in four databases, Pubmed, Web of Sciences, Scopus, and Cochrane, for the eligible articles until January 25, 2022 (Table 1). This systematic review is registered on the PROSPERO international prospective register of systematic reviews (CRD42022370010) and was conducted based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The terms used to search papers for this review were related to cancer, immune system, and exercise (Table 1). All obtained articles were stored in a Microsoft Excel file.

2.1. Search strategy

Table 1 - Database keywords

Databases	**Keywords**
PubMed	((Exercise) OR (Physical Activity)) AND ((cancer) OR (tumour) OR (tumor)) AND ((NK cells) OR (Lymphocytes) OR (Immune) OR (Macrophages) OR (T Cells) OR (CD4) OR (CD8) OR (CD3) OR (CD56)) with randomized controlled trial and clinical trial filter
Web of Science	TS=(Exercise OR Physical Activity) AND TS=(cancer OR tumour OR tumor) AND TS=(NK cells OR Lymphocytes OR Immune OR Macrophages OR T Cells OR CD4 OR CD8 OR CD3 OR CD56)
Scopus	ALL ((exercise OR physical AND activity) AND (cancer OR tumour OR tumor) AND (nk AND cells OR lymphocytes OR immune OR macrophages OR t AND cells OR cd4 OR cd8

	OR cd3 OR cd56)) AND (LIMIT-TO (DOCTYPE , "ar")) with article filter
Cochrane	(Exercise OR Physical Activity) AND (Cancer OR Tumour OR Tumor) AND (NK cells OR Lymphocytes OR Immune OR Macrophages OR T Cells OR CD4 OR CD8 OR CD3 OR CD56)

2.2. Inclusion and exclusion criteria

For the inclusion and exclusion criteria, the PICOS structure was adopted: **Population (P):** cancer patients or survivors; **Intervention (I):** studies that involved exercise interventions; those including multiple component interventions such as exercise and diet were excluded; **Comparator (C):** no comparison group was defined; **Outcome (O):** the outcomes reported must be related to the immune system (CD4, CD8, CD56, NK cells, monocytes, lymphocytes); **Study design (S):** randomized controlled trials (RCTs) written in English.

2.3. Study Selection and Data Extraction

The eligible studies were assessed by two authors (EO and IC) according to inclusion and exclusion criteria by evaluating the titles and abstracts of each paper. In cases where there was insufficient data to evaluate the article, the full-text version was retrieved. The reference lists of each eligible study were manually checked for additional papers. Details from each eligible article, including article identification, type of cancer, treatment, the number of participants and their ages, exercise intervention/duration, and results were extracted.

2.4. Quality assessment

Each included article's methodological quality was evaluated using the 'Physiotherapy Evidence Database (PEDro) scale'(de Morton, 2009), which is

considered to be a valid method to measure the methodological quality of clinical trials (de Morton, 2009). This tool assesses 11 items related to the study's internal validity and statistical reporting, however, the first item – eligibility criteria – is excluded from the final score (Paci et al., 2022). Therefore, the scale only evaluates 10 items: random allocation, concealed allocation, similarity at baseline, subject blinding, therapist blinding, assessor blinding, >85% follow-up for at least one key outcome, intention-to-treat analysis, between-group statistical comparison for at least one key outcome, and point and variability measures for at least one key outcome (de Morton, 2009). Items were rated as "1" if the criterion was satisfied. If there was a possibility that the criterion was not satisfied, a point should not be awarded, and a score of "0" should be attributed. If the trial scores below 5/10, it is considered to be of low quality; if the score is 6-8/10 it is considered to be of good quality and if it is 9-10 then the trial is considered to be of excellent quality (Cashin & McAuley, 2020).

3. Results

3.1. Study Process

The initial literature search identified 3833 articles, of which 462 were duplicated and 3114 were excluded after screening the title. The full text of 257 articles was screened and 249 studies were excluded due to not meeting the inclusion/exclusion criteria. After reviewing the references of eligible papers, 3 additional articles were included. A total of 11 investigations were included in this review, accounting for a total of 588 cancer patients. A PRISMA diagram is shown in Figure 1.

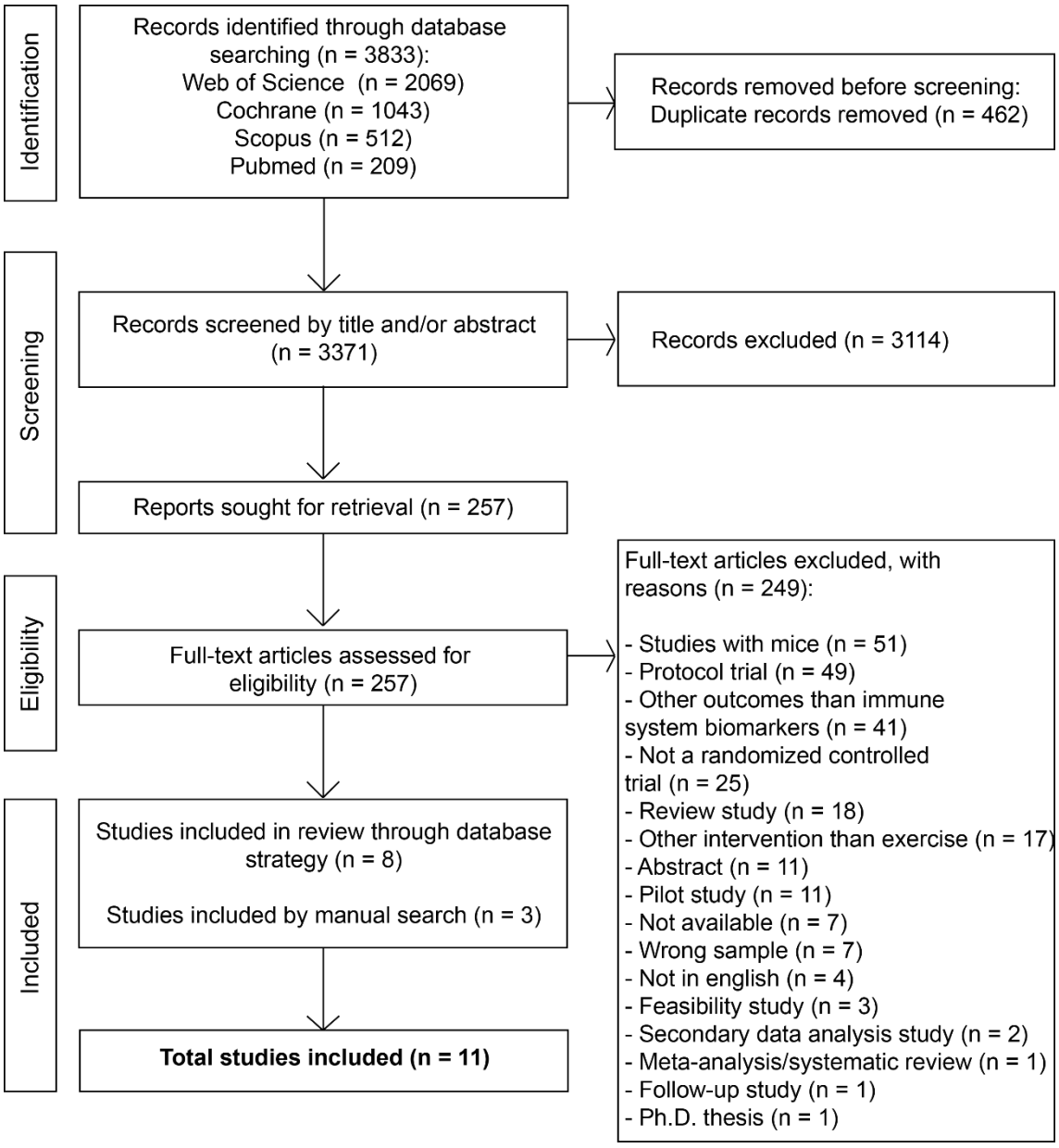

Figure 3 - PRISMA flow diagram

3.2. Characteristics of Included Investigations

Of the eleven investigations, six included only BC, one included Non-Hogkin lymphoma, two non-small cells lung cancer (NSCLC), one prostate carcinoma, and one Synovial sarcoma, Ewing sarcoma, osteosarcoma, Burkitt lymphoma, neuroblastoma, Hodgkin lymphoma, diffuse large B-cell lymphoma, T-cell lymphoblastic lymphoma, and ganglioneuroblastoma.

3.3. Methodological Quality of Included Investigations

The quality of the included investigations assessed by the PEDro scale varied from 5 to 10 points with an overall 6.9 points. Only one investigation achieved the perfect score of 10/10, one scored 9/10, one attained 8/10, three studies achieved 7/10, three studies scored 6/10, and two attained 5/10. Nine investigations were considered with moderate to high quality since they scored 6 or more points and the other two with low quality.

Table 2 - Methodological Quality Assessment of the included investigations

Author	1	2	3	4	5	6	7	8	9	10	11	Total
(Fairey, A., et al. 2005)	1	1	1	1	1	1	1	1	1	1	1	10
(Schmidt, T., et al. 2018)	1	1	1	1	1	1	1	0	1	1	1	9
(Hagstrom, A., et al. 2016)	1	1	1	1	0	0	1	1	1	1	1	8
(Hojan, K., et al. 2016)	1	1	1	1	0	0	0	1	1	1	1	7
(Fiuza-Luces, C., et al. 2017)	1	1	1	0	0	1	1	0	1	1	1	7
(Ligibel, J., et al. 2019)	1	1	0	1	0	0	1	1	1	1	1	7
(Liu, J., et al. 2015)	1	1	1	1	0	0	0	0	1	1	1	6
(Zimmer, P., et al. 2014)	1	1	0	1	0	0	1	1	1	0	1	6
(Mijewel, S., et al. 2020)	1	1	0	1	0	0	1	0	1	1	1	6
(Wang, R., et al. 2013)	1	1	0	1	0	0	0	0	1	1	1	5
(Nieman, D., et al. 1995)	1	1	0	1	0	0	0	0	1	1	1	5

3.4. Results of Individual Investigations

Studies including aerobic, resistance, and/or combined training protocols were the most common interventions, making it 9 out of the 11 investigations included (Fairey et al., 2005; Fiuza-Luces et al., 2017; Hagstrom et al., 2016; Hojan, 2016; Ligibel et al., 2019; Mijwel et al., 2020; Nieman et al., 1995; Schmidt et al., 2018; Zimmer et al., 2014).

In general, these interventions included activities such as walking, running, and cycling, as well as machine-based and free weights exercises.

The time of the exercise intervention varied across the studies, with only one study having an acute exercise intervention (30 minutes) and the longest intervention having 17 weeks. Also, the time/type of treatment was not the same. There was one study with androgen deprivation therapy, four studies with cancer survivors, one study that included patients scheduled for surgery, four studies performed in those undergoing chemotherapy (CTx) (neoadjuvant, adjuvant, or planned to receive adjuvant CTx), and one study after patients were submitted to surgery, CTx and/or radiation.

3.4.1. Aerobic Training Intervention

Considering the interventions with an aerobic approach (Fairey et al., 2005; Mijwel et al., 2020; Schmidt et al., 2018; Zimmer et al., 2014), it can be observed that only one of the four studies had between-group results regarding outcomes related to the immune system – intervention and control group – favoring the intervention group (Fairey et al., 2005). Further details on which biomarkers were affected by the exercise intervention are reported in Table 3.

3.4.2. Resistance Training Intervention

Regarding the resistance training interventions, only two of the RCTs included this type of intervention (Hagstrom et al., 2016; Schmidt et al., 2018). Both studies showed only within-group results, as disclosed in full detail in table 3.

3.4.3. Combined Training Intervention

In this systematic review, out of the 11 studies, 5 of them included combined training interventions (Fiuza-Luces et al., 2017; Hojan, 2016; Ligibel et al., 2019; Mijwel et al., 2020; Nieman et al., 1995). Two of them did not demonstrate any significant results (Fiuza-Luces et al., 2017; Nieman et al., 1995). One of them showed between-group results (Mijwel et al., 2020) regarding the concentration of thrombocytes and the other two investigations (Hojan, 2016; Ligibel et al., 2019) did not show between-group results.

3.4.4. Tai Chi Training Intervention

Two articles included Tai Chi as the exercise intervention (Liu et al., 2015; Wang et al., 2013). The earliest study reported only within-group results (Wang et al., 2013), whereas Liu et al. (Liu et al., 2015) demonstrated between and within-group results, improving the markers related to the immune system, favoring the intervention group.

Table 3 - Results of the included studies

First Author, Year	Cancer	Intervention	Sample size (age, years)	Treatment	Collection Methods and Endpoints	Outcomes	
						Within	**Between**
Schmidt, T. et al. 2018	Breast (patients)	**12-week** **G1**: 60 min, 2x/wk; 1 set of 20 reps with a hypothetical 50% of the maximum weight **G2**: 60 min, 2x/wk; indoor bike, 10 min warm-up, 25-30 min, and 5 min cool-down; level 11-14 on Borg scale **G3**: Usual care	**N**: 67 **G1**: 21 (53y) **G2**: 20 (56y) **G3**: 26 (54y)	Adjuvant CTx	Time points: • Baseline • 12 weeks • End of the intervention Collection methods • Flow cytometry • BD Multitest 6-color TBNK (M6T) Reagent with BD Trucount Beads	**CD3+ T Cells** • CD3+ T lymphocytes: G1: ↓; G2: ↓↓; G3: ↓↓ • Subset αβ T: G1: ↓; G2: ↓↓; G3: ↓↓ • Subset γδ T: G1:Ø; G2: ↓; G3: ↓ CD8+ T: G1: Ø; G2: ↓; G3: ↓ CD4+ T: G1: ↓↓; G2: ↓↓; G3: ↓↓ NK (CD16/CD56): G1: ↓; G2: ↓↓; G3: ↓↓ B cells (CD19): G1: ↓↓; G2: ↓↓; G3: ↓↓	Differences between arms were not significant
Fairey, A., et al. 2005	Breast (survivors)	**15-weeks** **G1:** 3x/wk; weeks 1-3: 15 min; increase 5 min every 3 wk; weeks 13-15: 35 min; recumbent or upright cycle ergometers; ~70–75% of peak oxygen consumption **G2:** Usual care	**N:** 52 (59y) **G1:** 24 (59y) **G2:** 28 (58y)	Completed Treatment	Time points: • Baseline • 15 weeks Collection methods: • Hemocytometer • Coulter STKS instrument • Flow cytometry • Immunofluorescence assay • ELISA kits		**NK cell cytotoxic activity** • 3.125:1 effector-to-target ratio: G1 > G2 • 6.25:1 effector-to-target ratio: G1 > G2 • 12.5:1 effector-to-target ratio: G1 > G2 • 25:1 effector-to-target ratio: G1 > G2

							• 50:1 effector-to-target ratio): G1 > G2 Total lytic units – G1 < G2
Wang, R. et al. (2013)	Non-small cell lung cancer (survivors)	**16 weeks** **G1:** 60 min, 3x/wk guided Tai Chi exercise; **G2:** Usual care	**N**: 27 **G1:** 13 (7 M and 6 W) (63.1y) **G2:** 14 (8 M and 6 W) (59.3y)	Treatment completed	Time points: • Baseline • End of the intervention Collection methods: • Flow cytometry	T1/T2: G1: Ø; G2 ↓ Tc1/Tc2: G1: Ø; G2 ↓ T1: G1: Ø; G2 ↓ TH1/TH2: G1: Ø; G2 ↓ Percentage of T2: G1 ↓; G2 ↑ Percentage of Tc2: G1 ↓; G2 ↑ Percentage of TH2: G1: Ø; G2 ↑	
Liu, J., et al. (2015)	Non-small cell lung cancer (survivors)	**16 weeks** **G1:** 60 min, 3x/wk, Tai Chi exercise **G2:** Usual care	**N:** 27 **G1:** 14 (8M and 6F) (62.6y) **G2:** 13 (7M and 6F) (60.5y)	Treatment completed	Time points: • Baseline • End of the intervention Collection methods: • Density gradient centrifugation • MTT cell proliferation kit • Flow cytometric analysis	PBMC proliferative capacity: G1: ↑; G2: Ø PBMC cytolytic/oncolytic activity against lung cancer cells A549: G1: ↑; G2: Ø	PBMC proliferation capacity: Ø Cytotoxicity of PBMCs: G1 > G2 Percentage of NK cells: G1 > G2 NKT and DC11c: G1 > G2
Hojan, K. (2016)	Prostate (patients)	**8 weeks** **G1:** 5x/wk MICT; 50-55 min: 30 min aerobic exercise (brisk walking, running indoors or on a treadmill, or cycling); 15 min resistance exercises, 2 sets of 8 reps at 70-75% RM **G2:** Usual care	**N:** 55 (68.5y) **G1:** 27 (67.4y) **G2:** 28 (69.9y)	Scheduled ADT	Time points: • Baseline • End of the intervention Collection methods: • BD Cytometric Bead Array (CBA) Enhanced Sensitivity Flex Set • Flow Cytometer	Most blood morphology parameters except monocyte levels: G1 ↓	Peripheral blood cell parameters: Ø
Nieman, D. (1995)	Breast (patients)	**8 weeks** **G1:** 60 min; 3x/wk; resistance training: 2 sets of 12 reps; aerobic	**N:** 12 **G1:** 6 (60.8y) **G2:** 6 (51.2y)	Undergone surgery, CTx, and/or radiation treatment	Time points: • Baseline • End of the intervention Collection methods:		NKCA and concentrations of circulating immune cells: Ø

		training: 75% heart rate max, walking on an indoor track for 30 min a session; **G2:** Usual care		within the previous four years	• Coulter STKS instrument		
Hagstrom, A., et al. (2016)	Breast (survivors)	**16-week** **G1:** 60 min; 3x/wk; resistance training: 3 sets of 8-10 reps at 8RM/80% of the 1RM; **G2:** Usual care	**N:** 39 (51.9y) **G1:** 20 (52.7y) **G2:** 19 (51.2y)	Treatment completed	Time points: • Baseline • End of the intervention Collection methods: • Fluorescence-activated cell sorting (FACS) • Multiparametric flow cytometry	Expression of TNF-α on their NK cells: G1 ↓; G2: Ø Expression of TNF-α on their NKT cells: G1 ↓; G2: Ø	
Fiuza-Luces, C. et al. (2017)	Synovial sarcoma Ewing sarcoma Osteosarcoma Burkitt lymphoma Neuroblastoma Hodgkin lymphoma Diffuse large B-cell lymphoma T-cell lymphoblastic lymphoma Ganglioneuroblastoma	**The mean duration of the exercise intervention: 17 (5) wks** **G1:** 3x/wk; 60- to 70-min; inhospital; Aerobic training: 30 mins (60 to 70% max HR); resistance training: 30 mins (2 to 3 sets of 8-15 reps) **G2:** Physiotherapy	**N:** 20 **G1:** 9 (11y) **G2:** 11 (12y)	Neoadjuvant CTx	Time points: • Baseline • End of the treatment • 2 months after the end of the treatment Collection methods: • Multiparametric flow cytometry • Polymerase chain reaction	Immune cell population counts and NK cell cytotoxicity: G1: Ø; G2: Ø	
Zimmer, P., et al. (2014)	Non-Hodgkin-Lymphoma	**30 min** **G1 and G3:** 30 min on a bicycle ergometer at moderate intensity (13–14 RPE) immediately after t1. **G2 and G4:** Usual care	**N:** 36 (26 patients and 10 healthy controls) **G1:** 14 **G2:** 12 **G3:** 5 **G4:** 5 **G1 and G2** (62.2y) **G3 and G4** (56.6y)	CTx	Time points: • Baseline • Baseline plus 1h Collection methods: • Ficoll-based density-gradient centrifugation protocol • Magnetic-activated cell sorting.	ΔCD8H4K5: G1: ↑; G3:↑	

Ligibel, J., et al. (2019)	Breast (patients)	**Meantime: 29.3 days** **G1:** 60-90 min, 2x/wk; 30-45 min of at least moderate-intensity aerobic training; 20 min of resistance training; 10 min cool down and stretching. Total (supervised and unsupervised): 220 min/wk, 40 min of strength, and 180 min of MICT **G2:** Mind-body control	**N:** 48 (52y)/ 46 **G1:** 26 (52.3y)/ 25 **G2:** 22 (53.1y)/ 21	Planning to undergo primary breast surgery	Time points: • Baseline • End of intervention Collection methods: • Radioimmunoassay • Automated chemistry analyzer • ELISA	Upregulation of pathways related to immunity: G1: ↑; G2: Ø	$FOXP3^+$ cells: Ø CD4+: Ø CD56+: Ø CD8+: Ø CD163+: Ø
Mijwel, S., et al. (2020)	Breast (patients)	**16 weeks** **G1**: 2x/wk, 60 minutes; 2-3 sets of 8–12 reps at @70-80% 1-RM; HIIT: 3x 3 min/1 min bouts at RPE 16 to 18 on a cycle ergometer; **G2**: 2x/wk, 60 minutes; 20 min of MICT, 13-15 RPE; same HIIT as in G1 **G3:** Usual care	**N:** 206 **G1:** 74 (52.7y) **G2:** 72 (54.4y) **G3:** 60 (52.6y)	Planned to receive adjuvant CTx	Time points: • Baseline • End of intervention Collection methods: • Not described.		**Thrombocyte concentration** • G1 > G2 (prior to 3rd CTx session) • G1 > G2 and G3 (prior to the 5th session) **Concentrations** of: • Hemoglobin: Ø • Lymphocyte: Ø • Neutrophil: Ø **Incidence of thrombocytopenia** • G1 and G2 < G3

4. Discussion

This systematic review provides an overview of the existent evidence of the effect of exercise on the immune system in cancer patients and survivors. Overall, this systematic review demonstrated that there are not many investigations with between-group results when compared to the control group made with exercise interventions such as aerobic, resistance, combined training, and Tai Chi and immune system-related markers on cancer patients and survivors. When it comes to the different types of exercise, only one of the studies that included aerobic exercise demonstrated between-group results; the studies with resistance training were in a small number and there were no between-group results reported; combined exercise interventions showed little benefits for the immune system, with only one study demonstrating between-group results; lastly, also only one of the two studies with Tai Chi intervention showed between-group results.

The current body of literature suggests that each bout of exercise has a direct effect on the circulation of immune cells and other components of the innate immune system, all of which if maintained periodically, have an anti-inflammatory and antioxidant effect with the capacity to modulate tumorigenesis and other diseases processes (Nieman & Wentz, 2019). During exercise, mechanisms like blood-flow-induced shear stress and adrenergic signalling are activated and cytotoxic immune cells are mobilized to the circulation (Christensen et al., 2018). For example, during a bout of exercise, the concentration of immune system cells in the circulation increases, with a more pronounced increase of NK cells than T and B cells (Idorn & Hojman, 2016), which could be explained since NK cells have the greater number of β-adrenergic receptors, making them the most sensible cells to the increased catecholamines' levels seen during exercise (Idorn & Hojman, 2016). The increase in catecholamines not only stimulates cells from the adaptive immune cell but also causes the recruitment of leucocytes into the peripheral blood, which means that the levels of

lymphocytes, neutrophils, and monocytes also increase (Thomas et al., 2017). Exercise has also been shown to augment vascularization and angiogenesis, which are correlated with enhanced blood perfusion, reduced tumor growth, better accessibility of circulating immune cells and treatment to tumors, and reduced intratumoral hypoxia, which has been shown to restrict NK cell-mediated lysis (Idorn & Hojman, 2016). Moreover, exercise also increases body temperature, which stimulates the circulation of immune cells since the diameter of the blood vessels increases and changes the vasculature of the tumors by inducing IL-6 trans-signalling, making them more receptive to the movement of cytotoxic T cells into the tumors (Hojman et al., 2017).

When it comes to the different types of exercise, literature suggests that aerobic exercise has positive effects on several immune markers, through the increases of both innate and adaptive immune cells (Goncalves et al., 2019). When looking at the animal mode, evidence shows that mice that voluntary wheel run have favourable effects on tumor onset and development in several tumor models and anatomical locations, which can be explained by the direct control of NK-cell trafficking (Pedersen et al., 2016). These changes imply a mobilization of NK cells and are dependent on epinephrine and an IL-6-dependent redistribution to the tumors (Pedersen et al., 2016). Resistance exercise also appears to have an impact on the immune system by increasing Th1, Th2, Th1/Th2 ratio, CD4+, and CD8+ (Lee & Jee, 2021). Experimental evidence with the effects of combined exercise is scarce, with no studies on cancer patients or survivors being found, however, when we observe the effects of this type of exercise on other health conditions, either no significant effects (Jin et al., 2018) or significant increases in CD4+ cells were found (Garcia et al., 2014). When we compare the expected exercise effects and the existent literature on the effects on the immune system, not all studies demonstrated the expected results but overall demonstrated that exercise has a positive impact on the immune system.

Exercise has the capacity to improve the function of the immune system, since it is able to reduce the negative effects of immune dysfunction brought by both aging and obesity, while also inducing changes in the adaptive and innate immune responses (Gustafson et al., 2021). This is verified both in healthy young and older people (Campbell & Turner, 2018). However, certain cancer treatments may be responsible for a temporarily weakened immune system (Fairey et al., 2002), such as radiotherapy (Wargo et al., 2015; Wasserman, 1989) or chemotherapy (Larsson et al., 2019). In these cases, exercise can counter some of these effects related to treatment by inducing a positive impact on the patient's immune system (Campbell & Turner, 2018). In the current systematic review, out of all 11 included investigations, 3 reported that exercise can cause significant alterations in immune system markers when compared to the control group (Fairey et al., 2005; Liu et al., 2015; Mijwel et al., 2020), showing significant alterations in several variables related to the immune system, thus improving the immunity in cancer patients.

When looking at the current body of evidence, much of the acute effects of exercise on the immune system stem from healthy individuals (Kurowski et al., 2022). Short bouts of exercise induce a rapid but transient increase in peripheral blood neutrophils numbers, with an ambiguous influence on neutrophil function (Campbell et al., 2009; Kurowski et al., 2022); a transient increase in peripheral monocytes (Kurowski et al., 2022); can modify dendritic cell's function and number (Kurowski et al., 2022); an increase in the number of T CD4+ and CD8+ (Campbell et al., 2009; Goncalves et al., 2019; Kurowski et al., 2022); increase mobilization of NK cells and CD8+ T lymphocytes (Nieman & Wentz, 2019); increase in CD3+ T cells (Goncalves et al., 2019). This systematic review only included one study with acute exercise (Zimmer et al., 2014), with results suggesting an increase in ΔCD8H4K5 in the intervention group, which means these results are in line with previous studies but are unable to demonstrate the rest of the expected outcomes.

When looking at the two Tai Chi studies, one analysed the effect of Tai Chi on immune balance (i.e. humoral and cellular immunity), demonstrating only within-group results (Wang et al., 2013). The other Tai Chi study assessed the effect on the immune function, with data reporting that Tai Chi did not have significant alterations on the proliferation capacity of PBMC but demonstrated significant differences in the cytotoxicity of PBMCs, in the percentage of NK cells and post-pre changes of NKT and DC11c between the exercise and control group (Liu et al., 2015). Previous evidence shows that, in a variety of cancers, the cytotoxicity of PBMCs is significantly reduced, when compared to non-cancer controls (Imai et al., 2000; Steinhauer et al., 1982), which does not mean that all types of cancer have the same behavior, such as colorectal and BC (Aparicio-Pagés et al., 1989). Physical exercise and Tai Chi may have the potential to stimulate PBMC cytotoxicity, which may improve anti-tumor cellular function (Fairey et al., 2002; Peters et al., 1994). Evidence on PBMC proliferation has not met a consensus effect, with some studies reporting that exercise (and/or weight loss) causes a decrease (Lin et al., 1993; Nieman et al., 1998), no effect (Hayes et al., 2003; Mitchell et al., 1996) or an increase (Liu et al., 2015).

However, not all studies showed significant alterations, since 8 out of the 11 included studies did not have substantial outcomes (Fiuza-Luces et al., 2017; Hagstrom et al., 2016; Hojan, 2016; Ligibel et al., 2019; Nieman et al., 1995; Schmidt et al., 2018; Wang et al., 2013; Zimmer et al., 2014). These results can be explained by several reasons, such as the methodological quality of the studies, as not all of the included studies are of high quality (only two of them are of high quality), and two of them are of low quality. A lower result on this assessment represents that there is a lack of internal validity. Also, the ages of the subjects included are between 50 to 60 years old, which could affect the impact of the benefits of exercise. Additionally, it could also be due to the different types of exercise, the intensity of exercise, the duration of each exercise session, or the duration of the exercise

intervention, all of which are known sources of interindividual variability, especially the exercise intensity which has a distinct impact on different cells of the immune system (Du & Wu, 2022) (Idorn & Hojman, 2016). Lastly, the trials deemed legible for this systematic review included several types of cancer, which is also a possible confounding factor. All these different factors can make generalizing these outcomes more difficult.

To the best of our knowledge, this is the first systematic review to evaluate the effects of exercise on immune system markers in cancer patients and survivors. One major strength of this review was the inclusion of only RCTs since this type of investigation is the most scientifically rigorous way of testing hypotheses and is considered to be the gold standard trial for assessing the efficacy of interventions. We also included every cancer stage or exercise intervention, which allows us to include a broader range of research and be more thorough. Also, this review looked at more than just the conventional forms of exercise (aerobic, resistance, or combined), we also assessed the effect of Tai Chi on immune system markers, which is a growing trend when it comes to exercise interventions in cancer patients. Nevertheless, this study is not without limitations. Firstly, there is little evidence on this topic, since the number of studies made to access the effects of exercise on immune system markers is scarce. There is also a lack of consensus on how and which markers to measure, which complicates the comparison between different papers. Moreover, the duration of the interventions between the different included studies was heterogenous, e.g., from 30 min to 17 weeks, which can complicate the generalization of the outcomes of different studies. Last, interventions that did not consist solely of exercise (e.g., exercise plus diet) were excluded, to focus just on the exercise effect in this population, which means that it is possible to be missing out on important data and information by not including these investigations. Although the last point may be a limitation, it can also be a strength in this study since diet can have an impact on the immune system and, thus, we would not be able to understand

if the immune system's alterations would derive from the exercise, from the diet or a combination of the two.

On a follow-up note, to this systematic review, it is possible to understand that several questions remain to be answered. Even though it is known that exercise is beneficial to the immune system, it is still necessary to understand which type, intensity, and duration of exercise has the best effects on the different immune system components for the different types of cancer and for the different stages of cancer, since each type and stage of cancer may have different effects on the immune system, therefore, providing detailed recommendations. More studies are also needed to understand the exercise effects on the different phases and types of treatment. Finally, more high-quality studies need to be done.

5. Conclusion

Based on the results of the included investigations, the number of investigations that showed that aerobic, resistance, combined, and Tai Chi exercise had an effect on the immune system in cancer patients and survivors are limited to three when compared to the control groups. Future research is needed which a focus on high-quality trials to have a better understanding of the impact that each type, intensity, and duration of exercise has on the different types and stages of cancer and the respective treatment.

Acknowledgment

None.

Funding

This work was conducted at the Interdisciplinary Center for the Study of Human Performance (CIPER), unit I&D 447 (UIDB/00447/2020), Faculty of Human Kinetics of the University of Lisbon, and supported by the Portuguese Foundation for Science and

Technology, the Portuguese Ministry of Science. IRC is supported by the Portuguese Foundation for Science and Technology (IRC: SFRH/BD/149394/2019)

References

A. LaPerriere, M. H. A., G. Ironson, A. Periy, P. McCabe, N Klimas, L. Helder,, & Fletche, N. S. a. M. A. (1994). Effects of Aerobic Exercise Training on Lymphocyte Subpopulations. *Int.J.Sports Med.* , *15*, S127—S130.

Aparicio-Pagés, N. M., Verspaget, H. W., Peña, S. A., & Lamers, C. B. (1989). Impaired local natural killer cell activity in human colorectal carcinomas. *Cancer Immunol Immunother*, *28*(4), 301-304. https://doi.org/10.1007/bf00205241

Ballard-Barbash, R., Friedenreich, C. M., Courneya, K. S., Siddiqi, S. M., McTiernan, A., & Alfano, C. M. (2012). Physical activity, biomarkers, and disease outcomes in cancer survivors: a systematic review. *J Natl Cancer Inst*, *104*(11), 815-840. https://doi.org/10.1093/jnci/djs207

Campbell, J. P., Riddell, N. E., Burns, V. E., Turner, M., van Zanten, J. J., Drayson, M. T., & Bosch, J. A. (2009). Acute exercise mobilises CD8+ T lymphocytes exhibiting an effector-memory phenotype. *Brain Behav Immun*, *23*(6), 767-775. https://doi.org/10.1016/j.bbi.2009.02.011

Campbell, J. P., & Turner, J. E. (2018). Debunking the Myth of Exercise-Induced Immune Suppression: Redefining the Impact of Exercise on Immunological Health Across the Lifespan. *Front Immunol*, *9*, 648. https://doi.org/10.3389/fimmu.2018.00648

Cashin, A. G., & McAuley, J. H. (2020). Clinimetrics: Physiotherapy Evidence Database (PEDro) Scale. *J Physiother*, *66*(1), 59. https://doi.org/10.1016/j.jphys.2019.08.005

Caspersen, C. R., Powell, K. E., & Christenson, G. M. (1985). Physical Activity, Exercise, and Physical Fitness: Definitions and Distinctions for Health-Related Research. *Public Health Reports*, *100*(2).

Cheung, M. N. B. a. L., W.T.Y. (2021). Effect of Tai Chi Chuan practice on immune system and pro-metastasis markers in early post-treatment breast cancer survivors. *World Journal of Advanced Research and Reviews*, *12*(1), 013-019. https://doi.org/10.30574/wjarr.2021.12.1.0472

Christensen, J. F., Simonsen, C., & Hojman, P. (2018). Exercise Training in Cancer Control and Treatment. *Compr Physiol*, *9*(1), 165-205. https://doi.org/10.1002/cphy.c180016

Courneya, K. S., Segal, R. J., Mackey, J. R., Gelmon, K., Reid, R. D., Friedenreich, C. M., Ladha, A. B., Proulx, C., Vallance, J. K., Lane, K., Yasui, Y., & McKenzie, D. C. (2007). Effects of aerobic and resistance exercise in breast cancer patients receiving adjuvant chemotherapy: a multicenter randomized controlled trial. *J Clin Oncol*, *25*(28), 4396-4404. https://doi.org/10.1200/JCO.2006.08.2024

de Morton, N. A. (2009). The PEDro scale is a valid measure of the methodological quality of clinical trials: a demographic study. *Australian Journal of Physiotherapy*, *55*(2), 129-133. https://doi.org/10.1016/s0004-9514(09)70043-1

Dethlefsen, C., Lillelund, C., Midtgaard, J., Andersen, C., Pedersen, B. K., Christensen, J. F., & Hojman, P. (2016). Exercise regulates breast cancer cell viability: systemic training adaptations versus acute exercise responses. *Breast Cancer Res Treat*, *159*(3), 469-479. https://doi.org/10.1007/s10549-016-3970-1

Djurhuus, S. S., Simonsen, C., Toft, B. G., Thomsen, S. N., Wielsoe, S., Roder, M. A., Hasselager, T., Ostergren, P. B., Jakobsen, H., Pedersen, B. K., Hojman, P., Brasso, K., & Christensen, J. F. (2023). Exercise training to increase tumour natural killer-cell infiltration in men with localised prostate cancer: a randomised controlled trial. *BJU Int*, *131*(1), 116-124. https://doi.org/10.1111/bju.15842

Du, F., & Wu, C. (2022). Review on the Effect of Exercise Training on Immune Function. *Biomed Res Int*, *2022*, 9933387. https://doi.org/10.1155/2022/9933387

Fairey, A. S., Courneya, K. S., Field, C. J., Bell, G. J., Jones, L. W., & Mackey, J. R. (2005). Randomized controlled trial of exercise and blood immune function in postmenopausal breast cancer survivors. *J Appl Physiol (1985)*, *98*(4), 1534-1540. https://doi.org/10.1152/japplphysiol.00566.2004

Fairey, A. S., Courneya, K. S., Field, C. J., & Mackey, J. R. (2002). Physical exercise and immune system function in cancer survivors: a comprehensive review and future directions. *Cancer*, *94*(2), 539-551. https://doi.org/10.1002/cncr.10244

Fiuza-Luces, C., Padilla, J. R., Valentin, J., Santana-Sosa, E., Santos-Lozano, A., Sanchis-Gomar, F., Pareja-Galeano, H., Morales, J. S., Fleck, S. J., Perez, M., Lassaletta, A., Soares-Miranda, L., Perez-Martinez, A., & Lucia, A. (2017). Effects of Exercise on the Immune Function of Pediatric Patients With Solid Tumors: Insights From the PAPEC Randomized Trial. *Am J Phys Med Rehabil*, *96*(11), 831-837. https://doi.org/10.1097/PHM.0000000000000757

Galvao, D. A., Taaffe, D. R., Spry, N., Joseph, D., & Newton, R. U. (2010). Combined resistance and aerobic exercise program reverses muscle loss in men undergoing androgen suppression therapy for prostate cancer without bone metastases: a randomized controlled trial. *J Clin Oncol*, *28*(2), 340-347. https://doi.org/10.1200/JCO.2009.23.2488

Gannon, G. A., Rhind, S. G., Shek, P. N., & Shephard, R. J. (2001). Differential cell adhesion molecule expression and lymphocyte mobilisation during prolonged aerobic exercise. *Eur J Appl Physiol*, *84*(4), 272-282. https://doi.org/10.1007/s004210000374

Garcia, A., Fraga, G. A., Vieira, R. C., Jr., Silva, C. M., Trombeta, J. C., Navalta, J. W., Prestes, J., & Voltarelli, F. A. (2014). Effects of combined exercise training on immunological, physical and biochemical parameters in individuals with HIV/AIDS. *J Sports Sci*, *32*(8), 785-792. https://doi.org/10.1080/02640414.2013.858177

Garcia, E., Becker, V. G., McCullough, D. J., Stabley, J. N., Gittemeier, E. M., Opoku-Acheampong, A. B., Sieman, D. W., & Behnke, B. J. (2016). Blood flow responses to mild-intensity exercise in ectopic vs. orthotopic prostate tumors; dependence upon host tissue hemodynamics and vascular reactivity. *J Appl Physiol (1985)*, *121*(1), 15-24. https://doi.org/10.1152/japplphysiol.00266.2016

Gleeson, M. (2006). *Immune function in Sport and Exercise*.

Goncalves, C. A. M., Dantas, P. M. S., Dos Santos, I. K., Dantas, M., da Silva, D. C. P., Cabral, B., Guerra, R. O., & Junior, G. B. C. (2019). Effect of Acute and Chronic Aerobic Exercise on Immunological Markers: A Systematic Review. *Front Physiol*, *10*, 1602. https://doi.org/10.3389/fphys.2019.01602

Gustafson, M. P., Wheatley-Guy, C. M., Rosenthal, A. C., Gastineau, D. A., Katsanis, E., Johnson, B. D., & Simpson, R. J. (2021). Exercise and the immune system: taking steps to improve responses to cancer immunotherapy. *J Immunother Cancer*, *9*(7). https://doi.org/10.1136/jitc-2020-001872

Hagstrom, A. D., Marshall, P. W., Lonsdale, C., Papalia, S., Cheema, B. S., Toben, C., Baune, B. T., Fiatarone Singh, M. A., & Green, S. (2016). The effect of resistance training on markers of immune function and inflammation in previously sedentary women recovering from breast cancer: a randomized controlled trial. *Breast Cancer Res Treat*, *155*(3), 471-482. https://doi.org/10.1007/s10549-016-3688-0

Hanahan, D. (2022). Hallmarks of Cancer: New Dimensions. *Cancer Discov*, *12*(1), 31-46. https://doi.org/10.1158/2159-8290.cd-21-1059

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell*, *144*(5), 646-674. https://doi.org/10.1016/j.cell.2011.02.013

Hayes, S. C., Rowbottom, D., Davies, P. S., Parker, T. W., & Bashford, J. (2003). Immunological changes after cancer treatment and participation in an exercise program. *Med Sci Sports Exerc*, *35*(1), 2-9. https://doi.org/10.1097/00005768-200301000-00002

Hiam-Galvez, K. J., Allen, B. M., & Spitzer, M. H. (2021). Systemic immunity in cancer. *Nat Rev Cancer*, *21*(6), 345-359. https://doi.org/10.1038/s41568-021-00347-z

Hojan, K., Kwiat Kowska-Borowczyk, E., Leporowska, E., Górecki, M., Ozga-Majchrzak. O., Milecki, T. and Milecki, P. (2016). Physical exercise for functional capacity, blood immune function, fatigue, and quality of life in high-risk prostate cancer patients during radiotherapy: a prospective, randomized clinical study. *European Journal of Physical and Rehabilitation Medicine*.

Hojman, P., Gehl, J., Christensen, J. F., & Pedersen, B. K. (2017). Molecular Mechanisms Linking Exercise to Cancer Prevention and Treatment. *Cell Metab*, *27*(1), 10-21. https://doi.org/10.1016/j.cmet.2017.09.015

Idorn, M., & Hojman, P. (2016). Exercise-Dependent Regulation of NK Cells in Cancer Protection. *Trends Mol Med*, *22*(7), 565-577. https://doi.org/10.1016/j.molmed.2016.05.007

Imai, K., Matsuyama, S., Miyake, S., Suga, K., & Nakachi, K. (2000). Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. *Lancet*, *356*(9244), 1795-1799. https://doi.org/10.1016/s0140-6736(00)03231-1

Jin, C. H., Rhyu, H. S., & Kim, J. Y. (2018). The effects of combined aerobic and resistance training on inflammatory markers in obese men. *J Exerc Rehabil*, *14*(4), 660-665. https://doi.org/10.12965/jer.1836294.147

Koelwyn, G. J., Quail, D. F., Zhang, X., White, R. M., & Jones, L. W. (2017). Exercise-dependent regulation of the tumour microenvironment. *Nat Rev Cancer*, *17*(10), 620-632. https://doi.org/10.1038/nrc.2017.78

Krause, P. J., Kavathas, P.B., and Ruddle, N.H. (2019). *Immunoepidemiology* (1 ed.). Springer. https://doi.org/10.1007/978-3-030-25553-4

Kurokawa , Y., Shinkai, S., Torii, J., Hino, S., and Shek, P.N. (1995). Exercise-induced changes in the expression of surface adhesion molecules on circulating granulocytes and lymphocytes subpopulations. *Eur J Appl Physiol*, *71*, 245-252.

Kurowski, M., Seys, S., Bonini, M., Del Giacco, S., Delgado, L., Diamant, Z., Kowalski, M. L., Moreira, A., Rukhadze, M., & Couto, M. (2022). Physical exercise, immune response, and susceptibility to infections—current knowledge and growing research areas. *Allergy*, *77*(9), 2653-2664. https://doi.org/10.1111/all.15328

Lambert, A. W., Pattabiraman, D. R., & Weinberg, R. A. (2017). Emerging Biological Principles of Metastasis. *Cell*, *168*(4), 670-691. https://doi.org/10.1016/j.cell.2016.11.037

Larsson, A. M., Roxa, A., Leandersson, K., & Bergenfelz, C. (2019). Impact of systemic therapy on circulating leukocyte populations in patients with metastatic breast cancer. *Sci Rep*, *9*(1), 13451. https://doi.org/10.1038/s41598-019-49943-y

Lee, J. K., & Jee, Y. S. (2021). Effect of Resistance Exercise on Acquired Immunocytes in Cancer Survivors: A Pilot Study. *Int Neurourol J*, *25*(Suppl 2), S96-105. https://doi.org/10.5213/inj.2142346.173

Ligibel, J. A., Dillon, D., Giobbie-Hurder, A., McTiernan, A., Frank, E., Cornwell, M., Pun, M., Campbell, N., Dowling, R. J. O., Chang, M. C., Tolaney, S., Chagpar, A. B., Yung, R. L., Freedman, R. A., Dominici, L. S., Golshan, M., Rhei, E., Taneja, K., Huang, Y., Brown, M., Winer, E. P., Jeselsohn, R., & Irwin, M. L. (2019). Impact of a Pre-Operative Exercise Intervention on Breast Cancer Proliferation and Gene Expression: Results from the Pre-

Operative Health and Body (PreHAB) Study. *Clin Cancer Res*, *25*(17), 5398-5406. https://doi.org/10.1158/1078-0432.CCR-18-3143
Lin, Y. S., Jan, M. S., & Chen, H. I. (1993). The Effect of Chronic and Acute Exercise on Immunity in Rats. *Int J Sports Med*, *14*(02), 86-92.
Liu, J. (2012). Effect of Tai Chi Exercise on Immune Function in Middle-aged and Elderly Women. *Journal of Sports Medicine & Doping Studies*, *02*(06). https://doi.org/10.4172/2161-0673.1000119
Liu, J., Chen, P., Wang, R., Yuan, Y., Wang, X., & Li, C. (2015). Effect of Tai Chi on mononuclear cell functions in patients with non-small cell lung cancer. *BMC Complement Altern Med*, *15*, 3. https://doi.org/10.1186/s12906-015-0517-7
McComb, S., Thiriot, A., Akache, B., Krishnan, L., & Stark, F. (2019). Introduction to the Immune System. *Methods Mol Biol*, *2024*, 1-24. https://doi.org/10.1007/978-1-4939-9597-4_1
McCullough, D. J., Nguyen, L. M., Siemann, D. W., & Behnke, B. J. (2013). Effects of exercise training on tumor hypoxia and vascular function in the rodent preclinical orthotopic prostate cancer model. *J Appl Physiol (1985)*, *115*(12), 1846-1854. https://doi.org/10.1152/japplphysiol.00949.2013
McCullough, D. J., Stabley, J. N., Siemann, D. W., & Behnke, B. J. (2014). Modulation of blood flow, hypoxia, and vascular function in orthotopic prostate tumors during exercise. *J Natl Cancer Inst*, *106*(4), dju036. https://doi.org/10.1093/jnci/dju036
McNeely, M. L., Parliament, M. B., Seikaly, H., Jha, N., Magee, D. J., Haykowsky, M. J., & Courneya, K. S. (2008). Effect of exercise on upper extremity pain and dysfunction in head and neck cancer survivors: a randomized controlled trial. *Cancer*, *113*(1), 214-222. https://doi.org/10.1002/cncr.23536
McTiernan, A. (2008). Mechanisms linking physical activity with cancer. *Nat Rev Cancer*, *8*(3), 205-211. https://doi.org/10.1038/nrc2325
Megha, K. B., & Mohanan, P. V. (2021). Role of immunoglobulin and antibodies in disease management. *Int J Biol Macromol*, *169*, 28-38. https://doi.org/10.1016/j.ijbiomac.2020.12.073
Mijwel, S., Bolam, K. A., Gerrevall, J., Foukakis, T., Wengstrom, Y., & Rundqvist, H. (2020). Effects of Exercise on Chemotherapy Completion and Hospitalization Rates: The OptiTrain Breast Cancer Trial. *Oncologist*, *25*(1), 23-32. https://doi.org/10.1634/theoncologist.2019-0262
Mitchell, J. B., Paquet, A. J., Pizza, F. X., Starling, R. D., Holtz, R. W., & Grandjean, P. W. (1996). The effect of moderate aerobic training on lymphocyte proliferation. *Int J Sports Med*, *17*(5), 384-389. https://doi.org/10.1055/s-2007-972865
Moyna, N. M., Acker, G.R., Weber, K.M., Fulton, J.R., Goss, F.L., Robertson, R.J., and Rabin, B.S. (1996). The effects of incremental submaximal exercise on circulating leukocytes in physically active and sedentary males and females. *Eur J Appl Physiol 74*, 211-218
Nehlsen-Cannarella, S. L., Nieman, D. C., Jessen, J., Chang, L., Gusewitch, G., Blix, G. G., and Ashley, E. . (1991). The Effects of Acute Moderate Exercise on Lymphocyte Function and Serum Immunoglobulin Levels. *Int. J. Sports Med.* , *12*, 391—398.
Nicholson, L. B. (2016). The immune system. *Essays Biochem*, *60*(3), 275-301. https://doi.org/10.1042/EBC20160017
Nieman, D. C., Cook, V. D., Henson, D. A., Suttles, J., Rejeski, W. J., Ribisl, P. M., Fagoaga, O. R., & Nehlsen-Cannarella, S. L. (1995). Moderate exercise training and natural killer cell cytotoxic activity in breast cancer patients. *Int J Sports Med*, *16*(5), 334-337. https://doi.org/10.1055/s-2007-973015
Nieman, D. C., Nehlsen-Cannarella, S. L., Henson, D. A., Koch, A. J., Butterworth, D. E., Fagoaga, O. R., & Utter, A. (1998). Immune response to exercise training and/or energy restriction in

obese women. *Medicine & Science in Sports & Exercise*, *30*(5), 679-686. https://journals.lww.com/acsm-msse/Fulltext/1998/05000/Immune_response_to_exercise_training_and_or_energy.6.aspx

Nieman, D. C., & Wentz, L. M. (2019). The compelling link between physical activity and the body's defense system. *J Sport Health Sci*, *8*(3), 201-217. https://doi.org/10.1016/j.jshs.2018.09.009

Oh, B., Bae, K., Lamoury, G., Eade, T., Boyle, F., Corless, B., Clarke, S., Yeung, A., Rosenthal, D., Schapira, L., & Back, M. (2020). The Effects of Tai Chi and Qigong on Immune Responses: A Systematic Review and Meta-Analysis. *Medicines (Basel)*, *7*(7). https://doi.org/10.3390/medicines7070039

Paci, M., Bianchini, C., & Baccini, M. (2022). Reliability of the PEDro scale: comparison between trials published in predatory and non-predatory journals. *Arch Physiother*, *12*(1), 10. https://doi.org/10.1186/s40945-022-00133-6

Patel, A. V., Friedenreich, C. M., Moore, S. C., Hayes, S. C., Silver, J. K., Campbell, K. L., Winters-Stone, K., Gerber, L. H., George, S. M., Fulton, J. E., Denlinger, C., Morris, G. S., Hue, T., Schmitz, K. H., & Matthews, C. E. (2019). American College of Sports Medicine Roundtable Report on Physical Activity, Sedentary Behavior, and Cancer Prevention and Control. *Med Sci Sports Exerc*, *51*(11), 2391-2402. https://doi.org/10.1249/MSS.0000000000002117

Pedersen, L., Idorn, M., Olofsson, G. H., Lauenborg, B., Nookaew, I., Hansen, R. H., Johannesen, H. H., Becker, J. C., Pedersen, K. S., Dethlefsen, C., Nielsen, J., Gehl, J., Pedersen, B. K., Thor Straten, P., & Hojman, P. (2016). Voluntary Running Suppresses Tumor Growth through Epinephrine- and IL-6-Dependent NK Cell Mobilization and Redistribution. *Cell Metab*, *23*(3), 554-562. https://doi.org/10.1016/j.cmet.2016.01.011

Peters, C., Lötzerich, H., Niemeier, B., Schüle, K., & Uhlenbruck, G. (1994). Influence of a moderate exercise training on natural killer cytotoxicity and personality traits in cancer patients. *Anticancer Res*, *14*(3a), 1033-1036.

Rock, C. L., Thomson, C. A., Sullivan, K. R., Howe, C. L., Kushi, L. H., Caan, B. J., Neuhouser, M. L., Bandera, E. V., Wang, Y., Robien, K., Basen-Engquist, K. M., Brown, J. C., Courneya, K. S., Crane, T. E., Garcia, D. O., Grant, B. L., Hamilton, K. K., Hartman, S. J., Kenfield, S. A., Martinez, M. E., Meyerhardt, J. A., Nekhlyudov, L., Overholser, L., Patel, A. V., Pinto, B. M., Platek, M. E., Rees-Punia, E., Spees, C. K., Gapstur, S. M., & McCullough, M. L. (2022). American Cancer Society nutrition and physical activity guideline for cancer survivors. *CA Cancer J Clin*, *72*(3), 230-262. https://doi.org/10.3322/caac.21719

Ruiz-Casado, A., Martín-Ruiz, A., Pérez, L. M., Provencio, M., Fiuza-Luces, C., & Lucia, A. (2017). Exercise and the Hallmarks of Cancer. *Trends Cancer*, *3*(6), 423-441. https://doi.org/10.1016/j.trecan.2017.04.007

Sasso, J. P., Eves, N. D., Christensen, J. F., Koelwyn, G. J., Scott, J., & Jones, L. W. (2015). A framework for prescription in exercise-oncology research. *J Cachexia Sarcopenia Muscle*, *6*(2), 115-124. https://doi.org/10.1002/jcsm.12042

Schmidt, T., Jonat, W., Wesch, D., Oberg, H. H., Adam-Klages, S., Keller, L., Rocken, C., & Mundhenke, C. (2018). Influence of physical activity on the immune system in breast cancer patients during chemotherapy. *J Cancer Res Clin Oncol*, *144*(3), 579-586. https://doi.org/10.1007/s00432-017-2573-5

Segal, R., Zwaal, C., Green, E., Tomasone, J. R., Loblaw, A., Petrella, T., & Exercise for People with Cancer Guideline Development, G. (2017). Exercise for people with cancer: a clinical practice guideline. *Curr Oncol*, *24*(1), 40-46. https://doi.org/10.3747/co.24.3376

Simpson, R. J., Kunz, H., Agha, N., & Graff, R. (2015). Exercise and the Regulation of Immune Functions. *Prog Mol Biol Transl Sci*, *135*, 355-380. https://doi.org/10.1016/bs.pmbts.2015.08.001

Steinhauer, E. H., Doyle, A. T., Reed, J., & Kadish, A. S. (1982). Defective natural cytotoxicity in patients with cancer: normal number of effector cells but decreased recycling capacity in patients with advanced disease. *The Journal of Immunology*, *129*(5), 2255-2259. https://www.jimmunol.org/content/jimmunol/129/5/2255.full.pdf

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*, *71*(3), 209-249. https://doi.org/10.3322/caac.21660

Thomas, R. J., Kenfield, S. A., & Jimenez, A. (2017). Exercise-induced biochemical changes and their potential influence on cancer: a scientific review. *Br J Sports Med*, *51*(8), 640-644. https://doi.org/10.1136/bjsports-2016-096343

Verronese, E., Delgado, A., Valladeau-Guilemond, J., Garin, G., Guillemaut, S., Tredan, O., Ray-Coquard, I., Bachelot, T., N'Kodia, A., Bardin-Dit-Courageot, C., Rigal, C., Perol, D., Caux, C., & Menetrier-Caux, C. (2016). Immune cell dysfunctions in breast cancer patients detected through whole blood multi-parametric flow cytometry assay. *Oncoimmunology*, *5*(3), e1100791. https://doi.org/10.1080/2162402X.2015.1100791

Wang, R., Liu, J., Chen, P., & Yu, D. (2013). Regular tai chi exercise decreases the percentage of type 2 cytokine-producing cells in postsurgical non-small cell lung cancer survivors. *Cancer Nurs*, *36*(4), E27-34. https://doi.org/10.1097/NCC.0b013e318268f7d5

Wargo, J. A., Reuben, A., Cooper, Z. A., Oh, K. S., & Sullivan, R. J. (2015). Immune Effects of Chemotherapy, Radiation, and Targeted Therapy and Opportunities for Combination With Immunotherapy. *Semin Oncol*, *42*(4), 601-616. https://doi.org/10.1053/j.seminoncol.2015.05.007

Wasserman, J., Blomgren, H., Rotstein, S., Petrini, B. and Hammarström, S. . (1989). Immunosuppression in irradiated breast cancer patients: in vitro effect of cyclooxygenase inhibitors. *Bull N Y Acad Med.*, *64*(1), 36-44.

Weinberg, R. A. (2014). *The Biology of Cancer* (2nd ed.). Garland Science.

WHO. (2021a). *The Global Cancer Observatory - Portugal*. Retrieved 19.09 from https://gco.iarc.fr/today/data/factsheets/populations/620-portugal-fact-sheets.pdf

WHO. (2021b). *The Global Cancer Observatory - World*. Retrieved 10.02 from https://gco.iarc.fr/today/data/factsheets/populations/900-world-fact-sheets.pdf

WHO, W. H. O. (2022). *Cancer*. Who.int; . Retrieved 10 February from https://www.who.int/news-room/fact-sheets/detail/cancer

Wolin, K. Y., Schwartz, A. L., Matthews, C. E., Courneya, K. S., & Schmitz, K. H. (2012). Implementing the exercise guidelines for cancer survivors. *J Support Oncol*, *10*(5), 171-177. https://doi.org/10.1016/j.suponc.2012.02.001

Zhang, Y.-J., Wang, R., Chen, P.-J., & Yu, D.-H. (2013). Effects of Tai Chi Chuan training on cellular immunity in post-surgical non-small cell lung cancer survivors: A randomized pilot trial. *Journal of Sport and Health Science*, *2*(2), 104-108. https://doi.org/10.1016/j.jshs.2013.02.001

Zimmer, P., Baumann, F. T., Bloch, W., Schenk, A., Koliamitra, C., Jensen, P., Mierau, A., Hulsdunker, T., Reinart, N., Hallek, M., & Elter, T. (2014). Impact of exercise on pro inflammatory

cytokine levels and epigenetic modulations of tumor-competitive lymphocytes in Non-Hodgkin-Lymphoma patients-randomized controlled trial. *Eur J Haematol*, *93*(6), 527-532. https://doi.org/10.1111/ejh.12395

Appendix

- PEDro scale

1. eligibility criteria were specified	no ❑	yes ❑	where:
2. subjects were randomly allocated to groups (in a crossover study, subjects were randomly allocated an order in which treatments were received)	no ❑	yes ❑	where:
3. allocation was concealed	no ❑	yes ❑	where:
4. the groups were similar at baseline regarding the most important prognostic indicators	no ❑	yes ❑	where:
5. there was blinding of all subjects	no ❑	yes ❑	where:
6. there was blinding of all therapists who administered the therapy	no ❑	yes ❑	where:
7. there was blinding of all assessors who measured at least one key outcome	no ❑	yes ❑	where:
8. measures of at least one key outcome were obtained from more than 85% of the subjects initially allocated to groups	no ❑	yes ❑	where:
9. all subjects for whom outcome measures were available received the treatment or control condition as allocated or, where this was not the case, data for at least one key outcome was analysed by “intention to treat”	no ❑	yes ❑	where:
10. the results of between-group statistical comparisons are reported for at least one key outcome	no ❑	yes ❑	where:
11. the study provides both point measures and measures of variability for at least one key outcome	no ❑	yes ❑	where:

The PEDro scale is based on the Delphi list developed by Verhagen and colleagues at the Department of Epidemiology, University of Maastricht (*Verhagen AP et al (1998). The Delphi list: a criteria list for quality assessment of randomised clinical trials for conducting systematic reviews developed by Delphi consensus. Journal of Clinical Epidemiology, 51(12):1235-41*). The list is based on "expert consensus" not, for the most part, on empirical data. Two additional items not on the Delphi list (PEDro scale items 8 and 10) have been included in the PEDro scale. As more empirical data comes to hand it may become possible to "weight" scale items so that the PEDro score reflects the importance of individual scale items.

The purpose of the PEDro scale is to help the users of the PEDro database rapidly identify which of the known or suspected randomised clinical trials (ie RCTs or CCTs) archived on the PEDro database are likely to be internally valid (criteria 2-9), and could have sufficient statistical information to make their results interpretable (criteria 10-11).

The PEDro scale should not be used as a measure of the "validity" of a study's conclusions. In particular, we caution users of the PEDro scale that studies which show significant treatment effects and which score highly on the PEDro scale do not necessarily provide evidence that the treatment is clinically useful. Additional considerations include whether the treatment effect was big enough to be clinically worthwhile, whether the positive effects of the treatment outweigh its negative effects, and the cost-effectiveness of the treatment. The scale should not be used to compare the "quality" of trials performed in different areas of therapy, primarily because it is not possible to satisfy all scale items in some areas of physiotherapy practice.

Last amended June 21st, 1999

Notes on administration of the PEDro scale:

All criteria **Points are only awarded when a criterion is clearly satisfied**. If on a literal reading of the trial report it is possible that a criterion was not satisfied, a point should not be awarded for that criterion.

Criterion 1 This criterion is satisfied if the report describes the source of subjects and a list of criteria used to determine who was eligible to participate in the study.

Criterion 2 A study is considered to have used random allocation if the report states that allocation was random. The precise method of randomisation need not be specified. Procedures such as coin-tossing and dice-rolling should be considered random. Quasi-randomisation allocation procedures such as allocation by hospital record number or birth date, or alternation, do not satisfy this criterion.

Criterion 3 *Concealed allocation* means that the person who determined if a subject was eligible for inclusion in the trial was unaware, when this decision was made, of which group the subject would be allocated to. A point is awarded for this criteria, even if it is not stated that allocation was concealed, when the report states that allocation was by sealed opaque envelopes or that allocation involved contacting the holder of the allocation schedule who was "off-site".

Criterion 4 At a minimum, in studies of therapeutic interventions, the report must describe at least one measure of the severity of the condition being treated and at least one (different) key outcome measure at baseline. The rater must be satisfied that the groups' outcomes would not be expected to differ, on the basis of baseline differences in prognostic variables alone, by a clinically significant amount. This criterion is satisfied even if only baseline data of study completers are presented.

Criteria 4, 7-11 *Key outcomes* are those outcomes which provide the primary measure of the effectiveness (or lack of effectiveness) of the therapy. In most studies, more than one variable is used as an outcome measure.

Criterion 5-7 *Blinding* means the person in question (subject, therapist or assessor) did not know which group the subject had been allocated to. In addition, subjects and therapists are only considered to be "blind" if it

could be expected that they would have been unable to distinguish between the treatments applied to different groups. In trials in which key outcomes are self-reported (eg, visual analogue scale, pain diary), the assessor is considered to be blind if the subject was blind.

Criterion 8 This criterion is only satisfied if the report explicitly states *both* the number of subjects initially allocated to groups *and* the number of subjects from whom key outcome measures were obtained. In trials in which outcomes are measured at several points in time, a key outcome must have been measured in more than 85% of subjects at one of those points in time.

Criterion 9 An *intention to treat* analysis means that, where subjects did not receive treatment (or the control condition) as allocated, and where measures of outcomes were available, the analysis was performed as if subjects received the treatment (or control condition) they were allocated to. This criterion is satisfied, even if there is no mention of analysis by intention to treat, if the report explicitly states that all subjects received treatment or control conditions as allocated.

Criterion 10 A *between-group* statistical comparison involves statistical comparison of one group with another. Depending on the design of the study, this may involve comparison of two or more treatments, or comparison of treatment with a control condition. The analysis may be a simple comparison of outcomes measured after the treatment was administered, or a comparison of the change in one group with the change in another (when a factorial analysis of variance has been used to analyse the data, the latter is often reported as a group □ time interaction). The comparison may be in the form hypothesis testing (which provides a “p” value, describing the probability that the groups differed only by chance) or in the form of an estimate (for example, the mean or median difference, or a difference in proportions, or number needed to treat, or a relative risk or hazard ratio) and its confidence interval.An additional criterion (criterion 1) that relates to the external validity (or “generalisability” or “applicability” of the trial) has been retained so that the Delphi list is complete, but this criterion will not be used to calculate the PEDro score reported on the PEDro web site.

Criterion 11 A *point measure* is a measure of the size of the treatment effect. The treatment effect may be described as a difference in group outcomes, or as the outcome in (each of) all groups. *Measures of variability* include standard deviations, standard errors, confidence intervals, interquartile ranges (or other quantile ranges), and ranges. Point measures and/or measures of variability may be provided graphically (for example, SDs may be given as error bars in a Figure) as long as it is clear what is being graphed (for example, as long as it is clear whether error bars represent SDs or SEs). Where outcomes are categorical, this criterion is considered to have been met if the number of subjects in each category is given for each group.