BIOMARKERS OF FATIGUE IN ONCOLOGY: A SYSTEMATIC REVIEW

Deveny Vanrusselt, MSc[1,2]; Charlotte Sleurs, PhD[1,2,3]; Mahnoor Arif, MSc[2]; Jurgen Lemiere, PhD [1,2]; Sabine Verschueren, PhD [4]; Anne Uyttebroeck, MD, PhD[1, 2]

[1] Pediatric Hematology and Oncology, University Hospitals Leuven, Leuven, Belgium
[2] Department of Oncology, KU Leuven, Leuven, Belgium
[3] Department of Social and Behavioral Sciences, Tilburg University, Tilburg, The Netherlands
[4] Department of Rehabilitation Sciences, KU Leuven, Leuven, Belgium

Corresponding author:

Deveny Vanrusselt

UZ Leuven, Pediatric Hematology-Oncology, Herestraat 49, 3000 Leuven, Belgium

Tel: 016/34.06.50, E-Mail: deveny.vanrusselt@kuleuven.be

Conflict Of Interest Statement

The authors declare no conflict of interest.

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ABSTRACT

Cancer-related fatigue (CRF) is a distressing side effect of cancer and treatment, affecting both patients during active treatment and survivors, negatively impacting quality of life. While its exact cause remains uncertain, various mechanisms such as immune dysfunction, HPA-axis dysfunction, and treatment toxicity are proposed. Inflammatory biomarkers of CRF have been explored in previous research, but non-inflammatory markers have not been comprehensively studied. This systematic review analysed 33 studies to identify non-inflammatory peripheral blood biomarkers associated with CRF. Promising markers included Hb, blood coagulation factors, BDNF, tryptophan, GAA, mtDNA, platinum, CA125, and cystatin-C. Inconsistent findings were observed for other markers like VEGF, leptin, and stress hormones. Most studies focused on adults. Research in pediatrics is limited. This review showed partial evidence for the inflammaging hypothesis (neurotoxicity due to neuro-inflammation) laying at the basis of CRF. Further research, especially in pediatrics, is needed to confirm this hypothesis and guide future biomarker studies.

Keywords: Biomarkers, Blood Biomarkers, Cancer-related Fatigue, Oncology, Review

1. INTRODUCTION

The amount of people surviving cancer has increased tremendously over the past decades due to the progress in cancer treatments, increased knowledge of individual risk factors and lifestyle, and better screening (1). Despite this positive evolution, cancer and its treatment can have major side effects, affecting the patients' lives both during active treatment and survivorship. Cancer-related fatigue (CRF) is one of the most common and distressing side-effects (2). Some studies report a prevalence of CRF in up to 85% of the cancer patients and survivors (3,4). CRF is a type of chronic fatigue that is unproportional to recently performed activity and does not disappear with rest or sleep (5). Its prevalence varies significantly based on the type of cancer, treatment, and method of fatigue assessment (6). A significant number of patients experience fatigue during treatment, often peaking at the end of active treatment, and sometimes even continuing until years after treatment. This prevents them from resuming their previous lifestyle and affects their daily life activities. Despite this impact, CRF often remains underdiagnosed and undertreated (7).

CRF is a multidimensional and complex symptom and several hypotheses have been formulated on the underlying pathological mechanisms (see figure 1). A first hypothesis suggests that an impaired immune response induces CRF (8). It is known that immunotherapy and chemotherapy can induce a hypersecretion of cytokines (i.e. cytokine release syndrome), which is related to fatigue and sickness symptoms (9). As cancer and its treatments are associated with increased inflammatory markers as well, it is possible that such release has similar effects. A second hypothesis suggests that it is dysregulation of the HPA-axis that lies at the base of CRF. The HPA-axis maintains the body's internal balance (homeostasis) and regulates the stress response. The HPA-axis function can be dysregulated by cancer-related stress, hormones produced by certain tumors, cancer treatment (chemotherapy, radiation therapy and immunotherapy) and certain medications (i.e. corticosteroids) (10–12). Both up- and downregulation of the HPA-axis can contribute to fatigue, although the mechanisms and causes differ. When the HPA-axis is dysregulated, altered (increased and decreased) cortisol levels can either directly or indirectly through acceleration of inflammation, lead to increased

fatigue (13–15). A third hypothesized cause of CRF is anemia. Anemia is a common complication in cancer patients, caused by the cancer directly, treatment, or other factors (e.g. a diet low in iron) (16). Chronic anemia leads to generalized hypoxia, resulting in severe fatigue (17). A fourth suggested cause of CRF can include reduced energy metabolism. Chemo- and radiotherapy can cause damage to the sarcoplasmic reticulum and mitochondria of cells, leading to a disrupted ATP production. This causes a decline in cellular (e.g. neuromuscular) efficiency (18,19). Such malfunctioning of the mitochondria, lowered ATP production and energy metabolism, also occurs in non-oncological patients with chronic fatigue syndrome (CFS) (20–22). A fifth hypothesis suggests endocrine dysfunction to be involved in CRF. Post-therapeutic thyroid dysfunction, such as hypothyroidism, can be caused by radio-, chemo-, immune-, or targeted therapy (23). Furthermore, a reduced production of sexual hormones testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) could appear after treatment (24). A sixth and last suggested cause of CRF might be other types of treatment-induced toxicity, including neurotoxicity (direct damage to neuron cell body and neurites or indirect glial damage), neuropathy (damage to sensory, motor, and autonomic nerves), cardiotoxicity or pulmonary toxicity (accumulation of intracellular reactive oxygen species (ROS)), each possibly occurring due to chemo- or radiotherapy (25–29). In a recent review, García-González et al. (2023) (30) concluded that inflammation, HPA-axis dysregulation, autonomic nervous system dysfunction and diet were the most frequently studied biological pathways potential underlying CRF in breast cancer survivors (BCS). However, CRF may exhibit distinctive characteristics specific to BCS, which may be less representative for other cancer types due to specific patient factors such as sex, hormonal influences, and the unique treatments associated with breast cancer.

In sum, even though multiple mechanisms have been hypothesized to cause CRF, it is crucial to emphasize that CRF does not solely rely on biological pathways. Instead, CRF results from a complex interaction of various factors: demographic factors (e.g. gender and age at diagnosis,…), medical factors (e.g. type of diagnosis, treatment, pre-existing health

conditions…), psychosocial factors (e.g. anxiety, depression, stress, social isolation and support,…) and behavioral factors (e.g. physical activity, sleep patterns,…) (14,31,32).

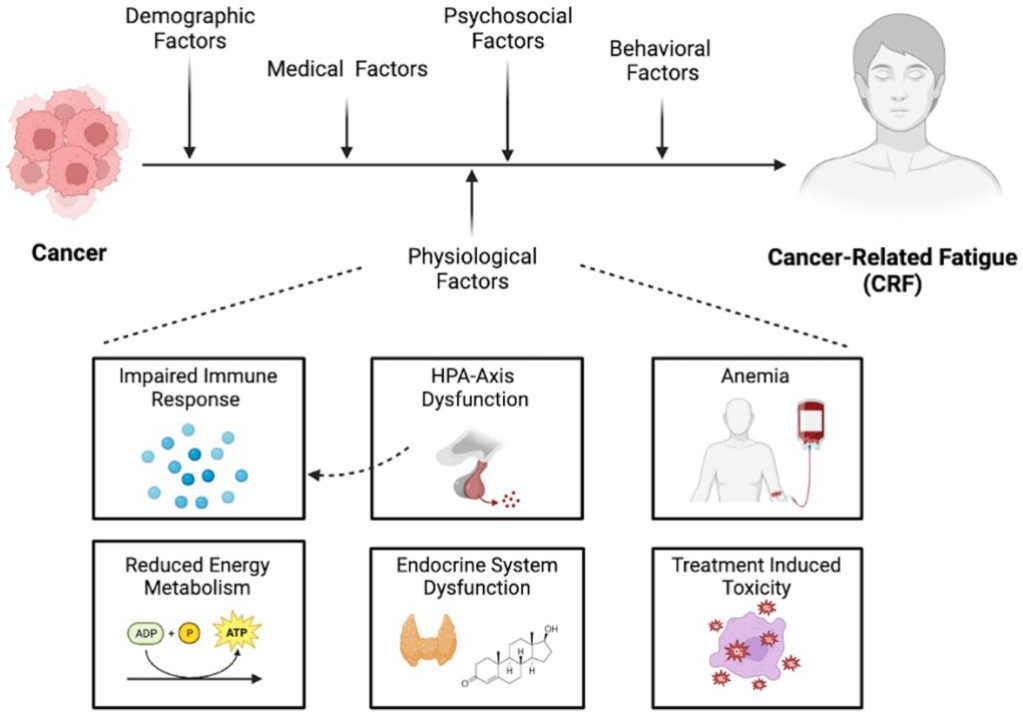


Figure 1: Overview of hypothesized mechanisms involved in cancer-related fatigue (CRF). The origin, duration and severity of CRF is an interplay of physiological factors, demographic factors (e.g. gender and age at diagnosis,…), medical factors (e.g. type of diagnosis and treatment, pre-existing health conditions,…), psychosocial factors (e.g. anxiety, depression, stress, social isolation and support,…) and behavioral factors (e.g. physical activity, sleep patterns,…). This figure was created with BioRender.

As reflected in the abovementioned studies, attempts were done to identify biomarkers that could contribute to the understanding of CRF. Most research has focused on an impaired immune response as potential cause of CRF. Inflammatory markers for CRF have earlier been summarized in reviews by Schubert et al. (2007) (33) and Bower et al. (2007) (34). Still, the exact etiology of CRF remains to be questioned. CRF might involve alternative pathways beyond just the inflammatory response. Therefore, compiling an overview of non-inflammatory markers becomes essential to gain more insights in the potential other biological mechanisms

involved. However, such a comprehensive overview has been lacking to date. Hence, the aim of this systematic literature review is to provide a comprehensive overview of non-inflammatory peripheral blood biomarkers associated with CRF.

2. METHODS

2.1 Search Strategy

This review was conducted in accordance with the updated guidelines for Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Page et al. (2021) (35)). A systematic literature search was conducted using PubMed and Embase on the 20th of March 2023. The search strategy was based on three main components: “biomarker”, “fatigue”, and “tumor”. Detailed search terms are presented in Appendix A. Only articles containing all 3 components were included. There were no limitations in terms of publication dates. The search was not narrowed to either pediatric or adult studies.

2.2 Study Selection

Articles were deemed eligible for inclusion if they met the following criteria: (1) original research studies, in (2) human cancer population (3) under treatment or after completion of treatment, investigating (4) fatigue as one of the main outcomes and (5) its potential blood biomarkers. Publications were excluded based on the following criteria: studies in other pathologies than cancer, drug trials for treatment of cancer, preclinical studies, trials for treatment of fatigue, reviews, studies with marker(s) not plasma/serum-based, studies with no biomarker(s), studies with biomarker(s) not related to fatigue, in vitro studies, case reports and studies investigating inflammatory markers of fatigue only. Non-English articles and those of which full text were not available, were also excluded. Screening of articles was conducted at three sequential stages: (1) titles, (2) abstracts and (3) full text. All publications were screened for eligibility by two team members (DV and MA) independently, based on abovementioned criteria, using the Rayyan system (36).

2.3 Data extraction

Data on authorship, publication year, number of participants, age, type of cancer, type of treatment, characteristics of controls, timing of assessment, measured biomarkers, types of fatigue assessment, and main findings for fatigue were extracted from each study.

2.4 Risk of bias

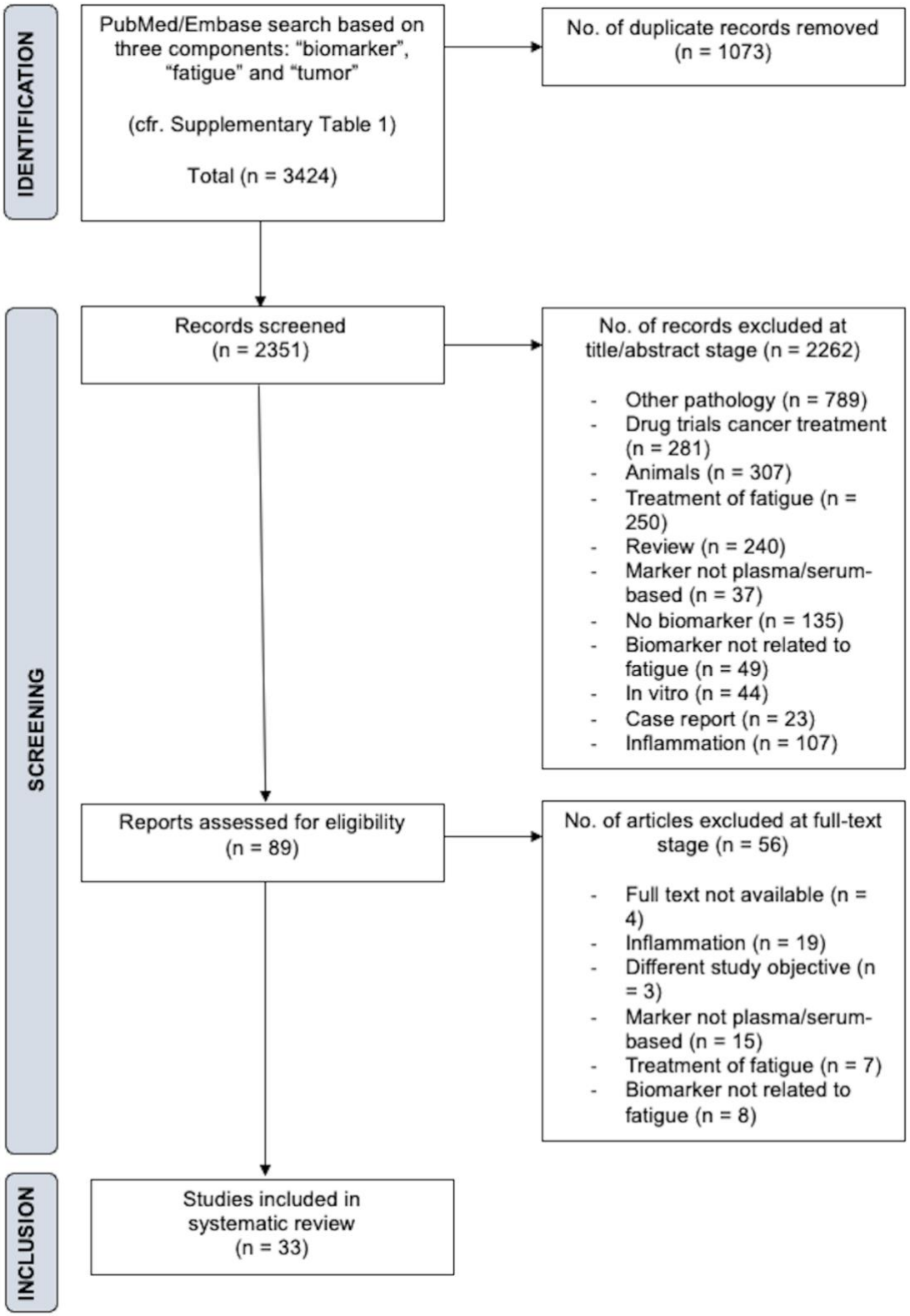
Risk of bias was assessed at study level using the Robvis tool (37). An overview of the risks of bias (incl. bias in sample size, bias in selection of patients, bias in measurement of the outcome and bias in selection of reported results) of each study and a general overview including all studies can be found in appendix B. A detailed summary on the specific risks of individual studies can be found in Appendix C.

3. RESULTS

3.1 Selection process

The search resulted in a total of 3424 articles that were imported into Rayyan. In total 2351 articles were screened for eligibility on title and abstract after deduplication in Rayyan. 2262 articles were excluded. A flowchart summarizing the selection process of the included articles is represented in Figure 2. All articles discussing inflammatory biomarkers only were excluded, since that exceeded the scope of this review. After title and abstract screening, 89 articles remained of which 56 publications were excluded at full text stage. Good inter-rater reliability was reached ($\kappa = 0.96$). After discussing the seven articles which were in conflict, 33 articles were included, and 100% agreement was reached.

Figure 2. PRISMA flowchart summarizing the selection process and reasons of exclusion.



3.2 Study and patient characteristics

All study details can be found in Table 1. Almost half of the studies (i.e. 16/33) were prospective longitudinal studies, observing participants prior to initiation of therapy until weeks or months after treatment (8,38–52). Nine studies were cross-sectional studies, with measurements at the start of therapy (n=1), during active treatment (n=4), or after completion of therapy (n=4) (24,53–61). Eight studies were case-control studies of which four studies compared fatigued to non-fatigued cancer patients (28,61–63). The remaining case-control studies were unique in comparing anemic to non-anemic cancer patients (5); cancer patients to healthy controls (64); cancer patients with normal versus dampened 24-hour rest/activity patterns (as measured using actigraphy) (65); and patients who went to treatment appointments alone compared to patients accompanied by family (66).

Regarding patient populations, most studies (n=11) were conducted in breast cancer patients or survivors (28,39,40,43,45,46,49,51,54,61,63). Eight studies were conducted in patients with hematological malignancies (24,52,55,56,58,59,62,66). Fewer studies were conducted in patients with colorectal cancer (n=3) (38,41,65), prostate cancer (n=3) (8,42,44), ovarian cancer (n=1) (64), lung cancer (n=1) (57). Six studies included each multiple types of diagnoses (5,47,48,50,53,60).

The age range of the included studies was 18-85 years with a study-specific average age between 50-70 years. Most studies were conducted in America (n=19) and Europe (n=9). Other studies were conducted in Asia (n=4) and Australia (n=1).

Most patients either underwent chemotherapy or radiotherapy only (n=21), or a combination of both with and without surgery (n=9), while the minority of patients were treated with high-dose chemotherapy in combination with Autologous Stem Cell Transplantation (HDT-ASCT) (n=3).

Different materials were used to measure self-report (or parent-report) fatigue. Most often questionnaires were used (n=31) (e.g. Functional Assessment of Cancer Therapy-Fatigue (FACT-F (8,24,38,44–46,53,57,59,60)), European Organization for Research and Treatment

of Cancer QLQ-C30 (EORTC QLQ-C30 (41,65)), Brief Fatigue Inventory (BFI (47,66)), Short-Form Health Survey (SF-36 (28,51,52)), Fatigue Symptom Inventory (FSI (54,55,63)), Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F (5,60)), Fatigue Questionnaire (FQ (55,56,62)), Multidimensional Fatigue Symptom Inventory Short Form (MFSI-SF (40,43)) or a combination of questionnaires (49,53,59). Most of these questionnaires focused on physical fatigue, apart from the MFSI-SF and the FQ also assessing cognitive fatigue. Only a few studies used alternative methods, including ecological momentary assessments (n=1) (42) or interviews (n=1) (50).

Peripheral blood markers of fatigue that resulted from the systematic search are categorized into six groups: (1) hematological markers (hemoglobin and blood coagulation factors), (2) hormones (gonadal hormones, leptin and stress hormones), (3) growth factors and neurotransmitters (Vascular Endothelial Growth Factor (VEGF), neurotrophins and tryptophan), (4) metabolic and nutritional markers (guanidine acetic acid (GAA) and mitochondrial DNA), (5) markers of oxidative stress and cardiac damage (malondialdehyde (MDA), 8-hydroxyguanosine (8-OH-dG), oxidized low-density lipoprotein (OxLDL), myeloperoxidase (MPO), cystatin-C and troponin-I (TnI)) and (6) cancer markers (cancer antigen 125 and platinum) as illustrated in Figure 3.

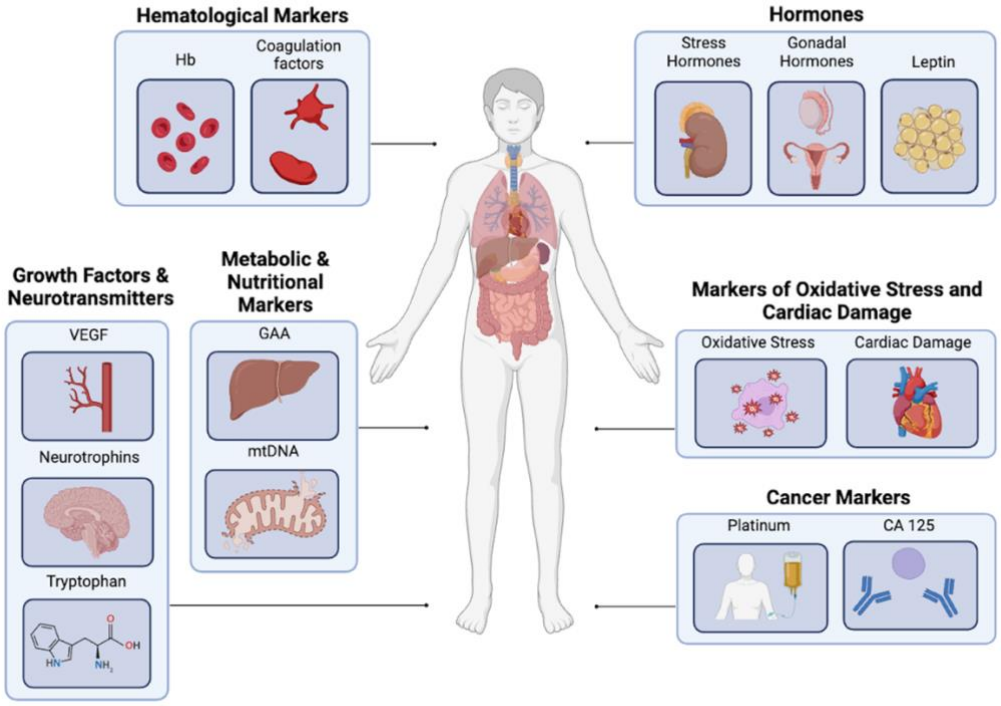


Figure 3: Peripheral blood markers included in this review. Figure was created with BioRender.

Note. CA 125=cancer antigen 125, GAA=guanidine acetic acid, Hb=hemoglobin, mtDNA=mitochondrial DNA, VEGF=vascular endothelial growth factor. Markers of oxidative stress include malondialdehyde (MDA), myeloperoxidase (MPO) and oxidized low-density lipoprotein (OxLDL). Markers of cardiac damage include 8-Hydroxyguanosine (8-OH-dG), Troponin-I (TnI), Cystatin-C and myeloperoxidase (MPO).

3.2.1 Hematological Markers

Regarding blood-specific markers, both hemoglobin (Hb) and coagulation factors were investigated as potential CRF markers. Hb is a protein that is crucial for the transport of oxygen in red blood cells and was investigated as a predictor of fatigue in ten studies (six longitudinal studies, three cross-sectional studies and one case-control study). All studies investigating Hb were performed in adult patients with different types of diagnoses. The findings showed mixed results, with the majority of studies (n=8) indicating a negative correlation between Hb and fatigue (correlations (r) ranging from e.g. -0.696 to -0.215 (5,38,48,50–52,59,60), while the minority did not find an association (n=2) (24,49).

Blood coagulation factors were also investigated as CRF markers. Blood coagulation factors are proteins present in the blood that play a crucial role in the process of blood clotting or coagulation. In case of damage to a blood vessel, the body initiates a series of complex reactions, known as the coagulation cascade, which could play a role in fatigue as it is known that alterations in blood coagulation factors (induced by the cancer and treatment) can affect blood flow and oxygen delivery to tissues, resulting in reduced oxygen supply (46,49). Blood coagulation factors were investigated in one longitudinal study in breast cancer patients. Wratten et al. (2004) found a negative correlation between fatigue and coagulation factors: Von Willebrand factor antigen, soluble thrombomodulin, tissue plasminogen activator and red blood cell count in breast cancer patients after surgery, prior to, during and after radiotherapy (46).

3.2.2 Hormones

Hormones that were investigated as biomarkers of fatigue thus far included gonadal hormones, leptin, and stress hormones. Gonadal hormones are known to mainly play a role in reproduction, sexual maturation and functioning of the neuro-endocrine axis. The relation between gonadal hormones and fatigue was explored in three studies (one longitudinal study, two cross-sectional studies). Hormones estradiol, testosterone, dehydroepiandrosterone sulfate (DHEAS), FSH and LH were investigated in patients with solid cancers and lymphomas. Mixed results were found for gonadal hormones. More specifically, weak negative correlations with fatigue were found for estradiol in women and for testosterone and DHEAS in males, in solid cancers (38,53). On the other hand, while elevated FSH, LH and reduced estradiol were encountered in females with lymphoma, no association with fatigue was demonstrated (55).
Furthermore, leptin was investigated as potential marker of CRF in two studies. Leptin is a protein hormone, predominantly made by adipose cells, and playing a role in the regulation of satiation (67). A significant negative correlation between leptin and fatigue was found in one prospective longitudinal study by Toh et al. (2019) in early-stage breast cancer patients undergoing chemotherapy (40). On the contrary, Kiecolt-Glaser et al. (2013) found

significantly higher leptin levels in fatigued breast cancer survivors compared to non-fatigued breast cancer survivors. Adiponectin was also investigated by Kiecolt-Glaser et al. (2013) but no differences were found between the fatigued and non-fatigued group (61).

Finally, more than half of the studies assessing hormones (i.e. 5/9), investigated the relation between stress hormones, more specifically cortisol, and fatigue, of which two additionally investigated (nor)epinephrine and ACTH. Three of the five studies investigating cortisol, were performed in breast cancer patients or survivors. Lower (morning) cortisol levels were found in fatigued breast cancer survivors compared to survivors with lower fatigue (39,63). By contrast, another study found cortisol, epinephrine and ACTH to be positively correlated with fatigue combined with pain and depression as symptom cluster in advanced breast cancer patients (54). This inconsistency was confirmed in another study which showed higher levels of fatigue in colorectal cancer patients with a dampened 24-hour rest/activity pattern (measured by actigraphy) compared to colorectal cancer patients with a normal 24-hour rest/activity pattern, but no differences in cortisol were found (65).

Regarding ACTH, findings also appear somewhat inconsistent, with for instance lower levels of ACTH in fatigued survivors of childhood ALL and lymphoma compared to non-fatigued survivors, in whom no differences in cortisol, epinephrine or norepinephrine were found (62). This latter study was one of the two exceptional studies performed in survivors of childhood cancer.

3.2.3 Growth factors and neurotransmitters

Regarding growth factors and neurotransmitters as potential biomarkers of fatigue, VEGF, neurotrophic factors, and tryptophan were investigated. VEGF is a signaling protein that plays a critical role in the growth of blood vessels (angiogenesis) and the maintenance of blood vessel function (vasculogenesis) in the body (68). Initial investigations involved two prospective longitudinal studies that examined the association between VEGF and fatigue, showing inconsistent results. On one hand, Himbert et al. (2019) found a positive correlation between VEGF factor D (VEGF-D) and fatigue in colorectal cancer patients before, six and

twelve months after surgery, using the EORTC QLQ-30 questionnaire (41). On the other hand, Holliday et al. (2016), found no relationship in prostate cancer patient undergoing external beam radiotherapy. This latter study implemented real-time ecological momentary assessment of fatigue via a wearable device (42).

Neurotrophic factors were also investigated as marker of CRF. Neurotrophic factors are a group of proteins that play a crucial role in the development, maintenance, and survival of neurons (nerve cells) in the nervous system. These proteins are responsible for promoting the growth, differentiation, and overall health of neurons, as well as assisting in the formation of synaptic connections between neurons (69).The relationship between neurotrophic factors and fatigue was investigated in two studies in radiotherapy-treated prostate cancer patients. Both studies yielded congruent outcomes in prostate cancer patients: high baseline soluble N-ethylmaleimide sensitive fusion attachment receptor-associated protein (SNAPIN) and decreasing brain-derived neurotrophic factor (BDNF) levels throughout external beam radiation therapy (EBRT) were associated with worsening of fatigue (8,44). In addition to SNAPIN and BDNF, Sass et al. (2020) found an upregulation of EV-associated Eotaxin, hsp27, IP-10, MIP-3α and soluble survivin in fatigued participants. No associations were found between glial-derived neurotrophic factor (GDNF) and fatigue in this study population (8).

Finally, the tryptophan-kynurenine pathway's in CRF was examined in three studies, yielding inconsistent findings. The tryptophan-kynurenine pathway is a biochemical pathway that involves the conversion of the amino acid tryptophan into various metabolites, primarily within the liver (70). The essential amino acid tryptophan is the precursor of melatonin and serotonin, essentials in mood and sleep and possibly explaining the coherence of mood, sleep and fatigue (71). The kynurenine pathway's metabolites also act as precursors for nicotinamide adenine dinucleotide (NAD) production, and so play a role in ATP production and energy homeostasis. Consequently, lower tryptophan levels can lead to low energy levels (56,72). Two studies demonstrated a positive relationship between tryptophan breakdown and fatigue in both lymphoma patients (56) and lung cancer patients (57). The effect in the latter population however only occurred in patients without antidepressant medications. On the contrary, Pertl

et al. (2013) found no indication of a link between markers associated with the kynurenine pathway activity and changes in fatigue in breast cancer patients at any timepoint throughout chemo- or radiotherapy (45).

3.2.4 Metabolic and Nutritional Markers

Concerning metabolic and nutritional markers related with fatigue, guanidine acetic acid (GAA) was investigated. GAA is a naturally occurring amino acid derivative that acts as a direct precursor of creatine, a vital element in the energy metabolism of muscle and nerve tissues (73). A positive correlation between fatigue and GAA was found in one case-control study by Zhang et al. (2023) in multiple myeloma patients undergoing chemotherapy (66). In addition to GAA, mitochondrial DNA (mtDNA) was investigated as marker of fatigue in one longitudinal study by Chae et al. (2017). Decreases in mtDNA in early-stage breast cancer patients (6 weeks after initiation of chemotherapy), were found to be associated with worsening of fatigue (43).

3.2.5 Markers of Oxidative Stress and Cardiac Damage

Regarding markers of oxidative stress and cardiac damage: myeloperoxidase, malondialdehyde, oxidized low-density lipoprotein, 88-Hydroxyguanosine, Troponin-I, cystatin-C and myeloperoxidase were investigated as potential CRF markers. Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS) or free radicals and the body's ability to neutralize or detoxify these harmful molecules. No relation was found between markers of oxidative stress myeloperoxidase, malondialdehyde and oxidized low-density lipoprotein and fatigue in survivors of childhood ALL in one study by Cheung et al. (2017) (58). Of all studies included in this review, this was one of the two studies performed in survivors of childhood cancer. Similar to the findings regarding oxidative stress, no associations were found between fatigue and markers of cardiac damage: 88-Hydroxyguanosine, Troponin-I, and myeloperoxidase in fatigued versus non-fatigued breast

cancer survivors. In the same study, higher levels of cystatin-C (hypothesized potential indirect marker of cardiac dysfunction (74,75)) were found to be positively associated with more fatigue (28).

3.2.6 Cancer Markers

In the final category, two studies investigated the relation between fatigue and cancer markers platinum and cancer antigen 125 (CA 125). Patients with various cancers treated with platinum-based chemotherapeutic agents oxaliplatin or cisplatin, with higher post-treatment levels of platinum, appeared to show a greater severity of fatigue in a longitudinal study by Zhang et al. (2021). Important to note that high levels of platinum were found, even when the drugs should have been metabolized and excreted from the body (47). Similar to platinum, the relationship between fatigue and CA 125 was investigated in one study. CA 125 is considered a tumor marker, produced by certain types of cancer cells and released into the bloodstream, which can be a marker of disease progression of certain cancers (76). One case-control study by Pickard-Holley et al. (1991) in ovarian cancer patients receiving chemotherapy and health controls, found weak-to-moderate positive relationships between levels of fatigue and CA 125 (64).

3.3 CRF, risk factors and link with biomarkers

Since previous research has shown that not all patients are equally susceptible to develop CRF, other factors besides the biological pathways, are in play. A first, and important suggested risk factor, making patients more prone to develop CRF, is the cancer itself. Most of the articles included in this review, showed more fatigue in patients with metastatic cancer than patients with localized cancer (38,48,50). Regarding biomarkers, no differences, however, were reported. Besides cancer stage, the subtype of cancer and its treatment are also influencing factors. Nevertheless, this review showed inconsistent results. The included longitudinal studies by Gascon et al. (2013), Geinitz et al. (2001), Himbert et al. (2019) and

Holliday et al. (2016) reported the highest fatigue levels in the active treatment phase (41,42,48,49). Wratten et al. (2004) and Von Ah et al. (2008) reported higher fatigue scores at diagnosis than during active treatment with radiotherapy (39,46) and Toh et al. (2019) reported the highest fatigue levels after chemotherapy. Regarding biomarkers, a significant decline in Hb levels was found throughout active treatment by Geinitz et al. (2001) (49), while no such time-effects were found in VEGF throughout chemotherapy or radiotherapy (41,42). Correlations between blood coagulation factors and fatigue were found to be significant at baseline only (46). Furthermore, a decrease in leptin was found after chemotherapy (40).

Concerning the type of treatment as suggested risk factor for CRF, only a few studies included different treatment types in their analysis. Bower et al. (2002) found no differences in fatigue between different breast cancer treatment regimens (63). Similarly, Von Ah et al. (2008) found no differences in CRF between different types of surgery (lumpectomy vs mastectomy) in this population (39). Consequently, differences in biomarkers between treatment regimens were not analyzed in these studies. Concerning other risk factors, anxiety and depression, assessed using questionnaires, were found to be strongly correlated with fatigue in multiple patient populations in this review (45,49,53,60). Regarding gender, results were inconsistent (48,55). Quality of sleep correlated with fatigue, whereas the number of hours slept, did not (49,60). Regarding performance status, this review showed an impaired performance status (or low Karnofsky score) to be a crucial component of CRF (24,48). However, none of these suggested risk factors were analyzed in regard to the biomarkers.

4. DISCUSSION

This systematic review presented the current state of peripheral non-inflammatory blood biomarkers as potential markers of fatigue in oncology. Indications of a potential relation between fatigue and markers, including Hb, blood coagulation factors, BDNF, GAA, tryptophan, mtDNA, platinum, CA125, and cystatin-C, were found, albeit for certain markers findings were limited to single studies. Despite several explorations of potential fatigue

biomarkers like VEGF, leptin, gonadal and stress hormones, their results appeared to remain inconsistent across studies. This underscores the necessity for further research to establish definitive conclusions regarding these markers.

4.1 Link with preclinical studies

Preclinical studies searching for biomarkers of fatigue have mainly been performed in murine models. In these studies, physical fatigue is assessed by means of treadmill running, a forced swim test, or a rotarod test (77,78), whereas the golden standard for assessment of cognitive fatigue is the Morris water maze or the Y maze test (79,80). Regardless of the methodological differences with human studies, meaningful parallels can be drawn (81). Even though the majority of human studies included in this review, showed associations between Hb and fatigue, preclinical studies have shown a lack thereof: Pouliot et al. (2009) found that fatigue was not related to anemia in their mouse model (82). Renner et al. (2016) also found no effects on Hb levels in mice with peripheral irradiation-induced fatigue (83). By contrast, hormonal effects were encountered in preclinical studies, with decreased cortisol levels leading to increased fatigue levels in a study by Huang et al. (2022) in an experimental mouse model after adrenal radiotherapy. Preclinical evidence was lacking for ACTH-CRF associations (84). These effects only partly support the human findings we have summarized. Only the neurotrophic findings seem to be consistent across species and studies sofar, albeit the number of these studies remains limited. More specifically, Wolff et al. (2020) induced fatigue-like behavior in mice using pelvic irradiation and showed lower BDNF in fatigued mice (80). The same group found that the BDNF polymorphism is involved in chemotherapy-induced fatigue in transgenic mice (85). Still, also in preclinical studies investigating the potential mechanisms of CRF in rodents, results are inconsistent. Most of the published preclinical studies concerning CRF to date, have focused on potential treatments, of which some could be promising. These included an thyrotropin-releasing hormone (86), a ganoderic acid for chemotherapy-induced fatigue in colon cancer (87), 1,25(OH)2D3 (the active metabolite of vitamin D) (88) or growth differentiation factor 15 (GDF15) (89).

4.2 The hypothesis of inflammaging and CRF

This review specifically focused on non-inflammatory markers of CRF, even though the most investigated markers of CRF to date are the inflammatory biomarkers. In the past, correlations were mainly found between fatigue and neutrophils/monocytes, NK cells, CD4+, IL-1β, IL-6, IL-1ra and neopterin (33,44). Despite the fact that the most research so far has been done on inflammatory markers of CRF, the exact role of cytokines in CRF, still remains under debate. Up- or downregulation in the production of pro- and anti-inflammatory cytokines can occur and can easily be dysregulated by all kinds of (small) infections already (e.g. a cold), which complicates standardized investigation and conclusions. Regarding potential interactions between inflammatory and non-inflammatory biomarkers, research has shown that central or peripheral inflammatory responses can cause dysregulation of glial and neuronal cells, ultimately leading to neural circuit and brain dysfunction (90). Such effects were recently shown explaining the post-covid-19-syndrome by Monje et al. (2022) (91). In analogy to this, CRF might similarly be caused by neurotoxicity following neuroinflammation due to the cancer and its treatment, which has been referred to as “inflammaging” (see figure 4). This could be caused by cancer treatment (chemotherapeutic agents or radiotherapy), hormonal changes due to the chemotherapy, genetic predisposition (genes related to neurotoxic vulnerability and neuronal repair mechanisms) and DNA damage (directly through chemotherapy or through oxidative stress) (92,93). This specific hypothesis is supported by the results of this review, where higher platinum levels were found to be related with higher fatigue levels in patients receiving cisplatin or oxaliplatin. It is known that platinum drugs can be neurotoxic (94). Furthermore, results showed low BDNF levels to be correlated with high fatigue levels. As mentioned above, BDNF plays an essential role in the survival of existing neurons, and growth and differentiation of new neurons and synapses. BDNF expression is influenced by stress, which, induced by e.g. cancer and cancer treatment, can lead to lower BDNF levels in the hippocampus (95). This diminishes synaptic plasticity and increases neuronal apoptosis, potentially contributing to fatigue. This review revealed a connection between cortisol and

fatigue; however, the exact direction of this relationship remains a topic of ongoing discussion. Nevertheless, previous research has already suggested that cortisol can also affect BDNF via gene regulation (prolonged high cortisol can cause epigenetic BDNF changes), hippocampal atrophy (chronic stress associates with smaller hippocampus and lower BDNF), and inflammation (96).

Prior research by Luine et al. (2013) suggested estrogen elevates BDNF in the prefrontal cortex and hippocampus, implying cooperation between estradiol and BDNF to enhance cognition (97). The interplay between estrogen and BDNF may be involved in CRF, possibly confirmed by the association found between fatigue and estradiol of the study by Vardy and colleagues (2014) included in this review (38).

In addition to the interactions among these abovementioned neuronal and endocrine-related components, possibly contributing to neuroinflammation, this review also presents evidence suggesting a possible role of tryptophan in the context of neuroinflammation. Tryptophan is known as the precursor of the neurotransmitter serotonin. Low serotonin levels are known to be associated with depression, anxiety and sleep problems (98). Nonetheless, earlier studies have demonstrated that the tryptophan metabolism can shift from the serotonin synthesis toward the synthesis of other potentially neurotoxic compounds (e.g. quinolinic acid) under the influence of stress hormones and proinflammatory cytokines (99) and as such contribute to neuroinflammation. In summary, this review provides partial evidence for CRF to be caused by “inflammaging” (neurotoxicity due to neuroinflammation), but more research is definitely needed to confirm this hypothesis.

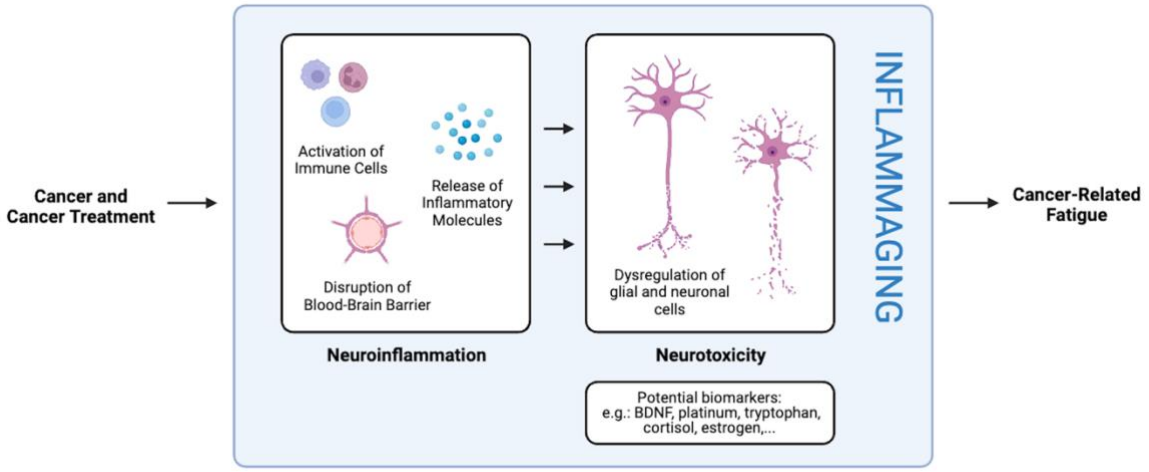


Figure 4: The inflammaging hypothesis underlying CRF. Figure was created with BioRender.

The cancer and cancer treatment can cause neuroinflammation (activation of immune cells, release of inflammatory molecules and disruption of the blood-brain barrier) either directly or through hormonal changes due to the chemotherapy, genetic predisposition (genes related to neurotoxic vulnerability and neuronal repair mechanisms) and DNA damage (directly through chemotherapy or through oxidative stress). In this hypothesis, neuroinflammation leads to neurotoxicity, potentially leading to CRF.

Even though inflammaging could also play a role in neurodevelopmental changes, almost all included studies investigated adult populations (either patients or survivors). Evidence of markers of CRF in childhood cancer is widely lacking. Only two studies included survivors of childhood ALL and lymphoma. So, the previously discussed hypothesis of neuroinflammation leading to neurotoxicity as potential cause of CRF, could differ in developing versus ageing brains. More specifically, vulnerabilities to altered neurodevelopment in case of childhood cancer, versus accelerated ageing mechanisms in middle aged adults need more and larger biomarker studies (100). Hence, the choice of markers of interest per study can depend on the investigated population, with neurodevelopmental and ageing markers as potential avenue in pediatrics and adult oncology, respectively.

4.3 Interventions for CRF and impact on biomarkers

The four most recommended treatments for CRF are physical exercise (e.g. stretching, yoga, combined exercise, aerobic exercise, resistance exercise,… (101)), psychological interventions, a combination of both or pharmaceutical intervention (102). In a recent review, Levesque et al. (2022) (103) summarized how contributors of fatigue are targeted by non-pharmaceutical interventions (e.g. Exercise counseling, Motivational interviewing, Cognitive behavior therapy, Community-based training,…). However, no link is made with biomarkers. Almost all published intervention studies that incorporated biomarkers in their designs so far, only focused on inflammatory markers. More specifically, reduced inflammation levels have been encountered by Serra et al. (2018) after whole-body resistance training programs (104). Van Vulpen et al. (2018) also showed changes in inflammatory markers related to changes in CRF after a 12-week resistance training program or an 18-week combined resistance and aerobic training program (105). Hiensch et al. found that changes in inflammatory markers mediated the exercise effects on both general and physical fatigue in patients with breast cancer (106). Besides inflammation as potential mechanism, this review showed that fatigue additionally correlated with hormones related to stress (cortisol, ACTH and epinephrine). In this perspective, Yuan et al. (2022) reported that mindfulness-based stress reduction therapy (MBSR), psychoeducational therapy (PE) and cognitive-behavioral therapy (CBT) were the most effective psychosocial interventions to reduce CRF in adults diagnosed with cancer, however no biomarkers were analyzed (107). Park et al. showed that mindfulness-based cognitive therapy reduced fatigue in breast cancer patients (108). Similar results were obtained by Kwekkeboom et al. (2018) (109) and Metin et al. (2019) (110), but also no biomarkers were analyzed. Currently, no published studies linking the reduction in fatigue by psychological interventions to stress hormone levels were found. Another, less commonly used method, being investigated for reducing CRF is light therapy. Johnson et al. (2016) showed that light therapy could reduce fatigue in cancer survivors (111). These findings were supported by Starreveld et al. (2018) in survivors of (non-) Hodgkin lymphoma, but no effect on cortisol and melatonin levels was found (112). Regarding physiological non-inflammatory changes, one intervention study of Repka et al. (2018) showed that a 10-week exercise intervention resulted

in an increased antioxidant capacity and decreased levels of fatigue (113). Since our review showed no correlations between oxidative stress and fatigue, these potential indirect relationships require additional investigation (94). Lastly, some studies investigated dietary changes to diminish fatigue. Zick et al. (2017) showed that an antioxidant-rich fatigue reduction diet could offer a non-toxic approach for managing persistent fatigue in breast cancer survivors (114). Baguley et al. (2021) also showed that the Mediterranean Diet (plant-based food and healthy fats) that dietary pattern changes could improve CRF and quality of life in prostate cancer patients, however, no biomarkers were investigated (115).

4.4 Limitations of the included studies and future directions

We should note several shortcomings of the studies included in this systematic review. First, and most important, is the heterogeneity of the included studies with regard to the assessment approach of fatigue. Of the 33 included articles, 31 studies used self-report or parent-report questionnaires to assess fatigue. One study assessed fatigue through interviews and one through an actiwatch score device for the rating of fatigue on a scale of 1–10 in real-time. Regarding the self-report questionnaires, fourteen different questionnaires were used. Not all questionnaires were fatigue-specific, leading to CRF as a subscore in some studies. Furthermore, not all questionnaires were uniquely developed for use in cancer patients and not all questionnaires were validated in oncology. Secondly, we need to note that there's a great variability in demographic variables, including age, type of cancer diagnosis, cancer stage, type of treatment and ethnicity. Most of the studies included in this review, recruited patients and/or survivors who were undergoing (or underwent) a particular type or combination of treatments, making it impossible to report about differences in fatigue levels between different treatments regimes. Hence, we need to be careful in generalizing the results of potential biomarkers of fatigue across different ages, diagnoses, treatment populations and treatment phases. It is uttermost important to mention that most of the included studies analysed fatigue and related biomarkers prior to therapy, during therapy or within 6 months after end of therapy. It is well known that cancer and cancer treatment cause fatigue. If we

want to capture the chronicity of CRF, which has been documented to potentially last for years after end of treatment, more studies covering a more extended post-treatment timeframe are needed. Only five studies analysed fatigue and biomarkers at more than 6 months after therapy (53,55,56,62,63). The small number of included studies and lack of subgroup-specific statistics, did not allow for meta-analysis. Further research is also needed to investigate the effect of gender, certainly, when looking into hormones as a biomarker. It is known that females are at risk for higher levels of fatigue (31). A third important limitation is the small sample size of most of the included studies, which ranged between 24 and 3492 participants (60% of the included studies had a sample size n<100). The statistical power may have been limited, certainly in case of multiple significance testing of questionnaire raw and domain scores and biomarkers. Further research in larger, homogenous groups is needed. A fourth and last limitation involves the sample collection for biomarker analysis and storage. Variability in results may be the result of differences in timepoint of sample collection and sample storage time. Regarding the systematic review, we need to note that it was based on the search of two databases (Pubmed and Embase) and limited to articles published in the English language. Even though we cannot exclude the possibility of missing additional individual studies, this was the first comprehensive systematic summary on potential non-inflammatory CRF markers.

5. CONCLUSION

To our knowledge this is the first systematic review providing an overview of potential non-inflammatory blood biomarkers of cancer-related fatigue. We identified biomarkers with significance on different time points in different populations receiving specific treatment regimes, providing direction for future studies. While some markers showed inconsistent findings (VEGF, leptin, gonadal and stress hormones), other markers were more stable and potentially promising for future CRF research (Hb, blood coagulation factors, BDNF, tryptophan, GAA, mtDNA, platinum, CA125 and cystatin-C). Still, we need to note that multiple markers were only limitedly investigated so far, and we are thus in need of more, homogenous and larger scale studies. Overall, these findings summarized the evidence for a potential role

of non-inflammatory biomarkers that could be related to inflammaging, and may offer initial guidance for designing upcoming research on CRF mechanisms. In future research it might be interesting to investigate the relationship between peripheral blood markers and fatigue, specifically incorporating neurotoxic markers, to look deeper into the hypothesis of inflammaging. In addition, while this review presented information on multiple peripheral blood markers linked to fatigue, future studies analyzing combinations of biomarkers will be useful and necessary to further unravel the underlying mechanisms of CRF.

Conflict Of Interest Statement

The authors declare no conflict of interest.

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Evidence Table – **Table 1**

Hematological Markers							
Authors	**Study design**	**Type of cancer**	**Type of treatment**	**Mean Age**	**Fatigue Assessment**	**Biomarkers**	**Findings**
Vardy et al. (2014) (38)	LNG	Localized CRC (n=291) Limited metastatic / recurrence CRC (n=72) HC (n=72)	Srg, chemo, RT or combination	Localized CRC: 58 yrs (23 - 75 range) Metastatic / recurrence CRC: 56 yrs (28 – 75 range) HC: 56 yrs (26 – 75 range)	FACT-F	Hb Prothrombin fragments D-dimers Estradiol	- Lower Hb and higher prothrombin fragments and D-dimers in patients with localized CRC compared to HC - Weak association between low Hb levels and high fatigue levels - Weak negative correlation between higher estradiol levels and fatigue
Gascón et al. (2013) (48)	LNG	Breast cancer (n = 129) Lung cancer (n = 133) Ovarian cancer (n = 52) Head and neck cancer (n = 33) Genitourinary cancer (n = 49) GI cancer (n = 194) Lymphoma (n = 16) Other (n = 117)	Without treatment (n=62) In treatment (n=605): chemo, RT, Immunotherapy, Hormonal therapy	60 yrs (20 – 89 range)	PERFORM Questionnaire	Hb	- Mild negative correlation between baseline Hb and patient perception of fatigue - Association between minimal increases or decreases in Hb of ≥1 g/dL and meaningful changes in patient-perceived fatigue
Booker et al. (2009) (59)	CS	MM (n=56)	Chemo	62 yrs (41 – 84 range)	FACT-F EORTC QLQ-C30	Hb	- Negative correlation between Hb and fatigue (Hb no longer significant predictor of fatigue after controlling for the effect of inflammation)
Geinitz et al. (2001) (49)	LNG	Breast cancer (n=41)	Adjuvant RT after BCS	54 yrs (34 – 77 range)	FAQ VAS	Hb	- No correlation between Hb and fatigue
Rodrigues et al. (2016) (60)	CS	Incurable solid tumors: GI tract (n=20) Genitourinary tract (n=4) Breast (n=8) Gynecologic tract (n=3) Lung (n=7) Other (n=9)	No therapy (except palliative RT)	64 yrs (33 – 85 range)	CFQ FACIT-F FACT-F	Hb	- Low Hb levels related to high fatigue levels
Olson et al. (2002) (50)	LNG	CRC (n=12) Lung cancer (n=17)	Chemo (n=14) Chemo- and RT (n=3)	37 – 80 range	Fatigue assessed	Hb	- Significant negative correlation between low Hb and fatigue during and after treatment for lung cancer

			RT-, chemo and srg (n=5) Srg and chemo (n=7)		through interviews			
Blair et al. (2008) (51)	LNG	Breast cancer (n=40)	Adjuvant chemo	48 yrs (28 – 70 range)	SF-36	Hb	-	Early low Hb levels predictive of high fatigue at 12 months
Wisløff et al. (2005) (52)	LNG	MM (n=745)	HDT-ASCT	62 yrs (28 – 87 range)	EORTC QLQ-C30	Hb	-	Hb and extent of skeletal disease strong predictors of fatigue
Cella et al. (2002) (5)	CC HC (n=1010) Nonanemic (n=113) Anemic (n =2369)	Nonmyeloid malignancy	Concomitant chemo	HC: 46 yrs Nonanemic: 55 yrs Anemic: 63 yrs	FACIT-F	Hb	-	Degree of anemia (mild, moderate or severe) predictive for the degree of fatigue
Dimeo et al (2004) (24)	CS	Hematological malignancies (n=71)	No chemo, RT or immune therapy for at least 3 months	51 yrs (21 – 72 range)	FACT-F	Hb Albumin	-	No correlation between fatigue and anemia and albumin
Wratten et al. (2004) (46)	LNG	Breast cancer (n = 52)	RT after BCS	56 yrs (31 – 74 range)	FACT-F	Blood coagulation factors	-	Negative correlation between fatigue and Von Willebrand factor antigen, soluble thrombomodulin, tissue plasminogen activator and red blood cell count

Hormones

Authors	Study design	Type of cancer	Type of treatment	Mean Age	Fatigue Assessment	Biomarkers	Findings
Vardy et al. (2014) (38)	LNG	Localised CRC (n=291) Limited metastatic / recurrence CRC (n=72) HC (n=72)	Srg, chemo, RT or combination	Localised CRC: 58 yrs (23 -75 range) Metastatic / recurrence CRC: 56 yrs (28 – 75 range) HC: 56 yrs (26 – 75 range)	FACT-F	Hb Prothrombin fragments D-dimers Estradiol	- Lower Hb and higher prothrombin fragments and D-dimers in patients with localized CRC compared to HC - Weak association between low Hb levels and high fatigue levels - Weak negative correlation between higher estradiol levels and fatigue

Knobel et al. (2000) (55)	CS	Lymphomas (n=33)	ABMT	39 yrs (18 – 59 range)	FQ EORTC QLQ-C30	FSH LH Estradiol Testosterone	- -	Gonadal dysfunction with elevated FSH, LH and reduced levels of estradiol in majority of women, but no association with fatigue. No association between fatigue and endocrine function.
Shafqat et al. (2005) (53)	CS	Lung (n=72) Breast (n=33) Lymphoma (n=32) Renal (n=13) Germ cell tumor (n=7) Other (n=17)	Chemo (n=118) RT (n=6)	58 yrs (20 – 83 range)	BFI FACT-F	DHEAS Testosterone	-	Weak negative correlation between fatigue scores and DHEAS levels and testosterone levels in male patients
Toh et al. (2019) (40)	LNG	Early-stage breast cancer (n = 136)	Adjuvant chemo	51 yrs	MFSI-SF	Leptin	-	A significant negative correlation between leptin and MFSI-SF score
Kiecolt-Glaser et al. (2013) (61)	CC	Stage 0- IIIC breast cancer survivors (n=162)	Srg, Chemo, RT, or a combination	51 yrs	RAND 36-item health survey	Leptin Adiponectin	- - -	Significantly higher leptin in fatigued group compared to non-fatigued group. No differences in adiponectin between fatigued and non-fatigued
Von Ah et al. (2008) (39)	LNG	Breast cancer (n=44)	Adjuvant chemo- and/or RT post-srg	52 yrs (38 – 77 range)	PFS Revised	Cortisol	-	Negative correlation between morning cortisol levels before, during and after chemotherapy and CRF
Zeller et al. (2014) (62)	CC cf (n=35) no-cf (n=52)	Survivors of childhood ALL (n=43) and Lymphoma (n=44)	Chemo	Cases: 32 yrs (22 – 50 range) Controls: 34 yrs (20 – 53 range)	FQ	ACTH Epinephrine Norepinephrine Cortisol	- -	Significant lower levels of ACTH in the cf group compared to the no-cf group No significant differences in plasma cortisol, epinephrine and norepinephrine between cf group and no-cf group
Bower et al. (2002) (63)	CC CF (n=20) No CF (n=20)	Breast cancer survivors (n=40)	Srg only (n=12) Srg + chemo (n=5) Srg + RT (n=13) Srg + chemo+ RT (n=11)	Cases: 57 yrs Controls: 58 yrs	FSI RAND 36-item health survey	Cortisol	-	Significant lower serum cortisol levels in fatigued survivors compared to non-fatigues survivors.
Thornton et al. (2010) (54)	CS	Recurrent and advanced stage breast cancer (n=104)	Srg, Chemo, RT, Hormonal Therapy or a combination	53 yrs (21 – 85 range)	FSI	Cortisol ACTH Epinephrine Norepinephrine	- -	Positive moderate correlations between PDF and cortisol and epinephrine Positive mild correlation between ACTH and PDF
Rich et al. (2005) (65)	CC Cases (n=40) Controls (n=40)	Metastatic CRC (n=80)	Chemo	Cases: 59 yrs (42 – 76 range) Controls: 60 yrs (36 – 76 range)	EORTC QLQ-C30	Cortisol	-	No significantly different mean serum cortisol concentrations between normal and dampened 24-hour rest/activity pattern group Higher fatigue levels in dampened rest/activity pattern group

Growth Factors and Neurotransmitters

Authors	Study design	Type of cancer	Type of treatment	Mean Age	Fatigue Assessment	Biomarkers	Findings
Himbert et al. (2019) (41)	LNG	CRC (n = 236)	(Neo)adjuvant therapy	63 yrs	EORTC QLQ-30	VEGFD	- Positive correlation between VEGF-D and fatigue
Holliday et al. (2016) (42)	LNG	Prostate cancer (n = 28)	EBRT	67 yrs	AW-S device for the rating of fatigue on a scale of 1–10 in real-time	VEGF	- No relationship between fatigue and VEGF
Saligan et al. (2016) (116)	LNG	Prostate cancer (n = 47)	EBRT	63 yrs (49 – 81 range)	FACT-F	BDNF GDNF SNAPIN	- Baseline SNAPIN and decreasing BDNF levels may influence worsening of fatigue during EBRT
Sass et al. (2020) (8)	LNG	Prostate cancer (n = 40)	EBRT	Fatigued and Non-fatigued: 67 yrs	FACT-F	Soluble and extracellular vesicle - associated markers BDNF	- Upregulation of EV-associated Eotaxin, hsp27, IP-10, MIP-3α and soluble survivin in fatigued participants - Positive correlation between BDNF and fatigue
Fossa et al. (2020) (56)	CS	Lymphoma survivors (n = 244)	HDT-ASCT	Fatigued: 56 yrs (25 – 76 range) Non-fatigued: 55 yrs (24 – 77 range)	FQ	Tryptophan	- Significantly lower tryptophan levels in both males and females with CF compared to non-fatigued survivors
Pertl et al. (2013) (45)	LNG	Breast cancer (n = 61)	Srg, chemo, RT hormonal therapy or combination	50 yrs	FACT-F	Tryptophan	- No evidence of role of KP activation in fatigue - No indication of link between KP activity markers and changes in fatigue over the treatment trajectory
Kurz et al. (2012) (57)	CS	Lung cancer NSCLC (n = 38) SLCC (n = 12)	Chemo (n=36) RT (n=8)	65 yrs	FACT-F	Tryptophan	- Positive associations between tryptophan breakdown and fatigue, but only in patients without antidepressant medications

Metabolic and Nutritional Markers

Authors	Study design	Type of cancer	Type of treatment	Mean Age	Fatigue Assessment	Biomarkers	Findings
Zhang et al. (2023) (66)	CC Accompanied (n=12)	MM (n=30)	Chemo	57 yrs	BFI	GAA	- Significant positive correlation between GAA and fatigue in MM patients

	Alone (n=18)						
Chae et al. (2018) (43)	LNG	Early-stage breast cancer (n = 108)	Adjuvant chemo	52 yrs	MFSI-SF	mtDNA in peripheral blood	- mtDNA decrease significantly associated with worsening of CRF

Markers of Oxidative Stress and Cardiac Damage

Authors	Study design	Type of cancer	Type of treatment	Mean Age	Fatigue Assessment	Biomarkers	Findings
Cheung et al. (2017) (58)	CS	Survivors of childhood ALL (n = 70)	Chemo	14 yrs	PedsQL-MFS (parent-reported or self-reported)	MDA MPO OxLDL	- No association between fatigue and biomarkers of oxidative stress
Vasbinder et al. (2022) (28)	CC CF (n=50) No CF (n=130)	Breast cancer survivors	RT	67 yrs	SF-36	8-OH-dG MPO Cystatin-C TnI	- Levels of cystatin-C positively associated with fatigue - No associations for 8-OH-dG, MPO, or TnI

Cancer Markers

Authors	Study design	Type of cancer	Type of treatment	Mean Age	Fatigue Assessment	Biomarkers	Findings
Zhang et al. (2021) (47)	LNG	Different types (n = 135)	Chemo (platinum-based)	Cisplatin: 56 yrs Oxaliplatin: 60 yrs	BFI	Platinum	- Higher incidence and greater severity of fatigue in patients with high platinum levels
Pickard-Holley et al. (1991) (64)	CC Cases (n=12) Controls (n=12)	Ovarian cancer	Chemo	Cases: 55 yrs (43 – 73 range) Controls: 50 yrs (24 – 86 range)	RFS	CA 125	- Positive weak-to-moderate relationships between levels of fatigue and CA 125

Abbreviations: yrs, years; LNG, longitudinal study; CC, case-control study; CS, cross-sectional study; CRC, colorectal cancer; HC, healthy controls; Srg, surgery; FACT-F, Functional Assessment of Cancer Therapy-Fatigue; PFS, Piper Fatigue Scale; Hb, hemoglobin; CRF, cancer related fatigue; ACTH, Adrenocorticotropic Hormone; DHEAS, dehydroepiandrosterone-sulfate; ABMT, autologous bone marrow transplantation; FSH, follicle-stimulating hormone; LH, luteinizing hormone; BFI, Brief Fatigue Inventory; FSI, Fatigue Symptom Inventory; FACIT, Functional Assessment of Chronic Illness Therapy; PDF, pain, depression and fatigue; FQ, Fatigue Questionnaire; MFSI-SF, Multidimensional Fatigue Symptom Inventory – Short form; EORTC QLQ-30, European Organization for Research and Treatment of Cancer QLQ-C30; VEGF, Vascular Endothelial Growth Factor; AW-S device, Actiwatch Score device; EBRT, external beam radiation therapy; BNDF, brain-derived neurotrophic factor; GDNF, glial-derived neurotrophic factor; SNAPIN, High soluble N- ethylmaleimide sensitive fusion attachment receptor-associated protein; HDT-ASCT, High dose chemotherapy followed by Autologous Stem Cell Transplantation; NSCLC, non- small cell lung cancer; SLCC, small cell lung cancer; BCS, breast conserving surgery; SF-36, 36-Item Short-Form Health Survey; RFS, Rhoten Fatigue Scale; CA125, Cancer antigen 125; ALL, acute lymphoblastic leukemia; PedsQL-MFS, Pediatric Quality of Life Inventory Multidimensional Fatigue Scale; MDA, malondialdehyde; MPO myeloperoxidase; OxLDL, oxidized low-density lipoprotein; GI, gastrointestinal; QoL, Quality of Life; FAQ, Fatigue Assessment Questionnaire; VAS, visual analog scale; CFQ, Chalder Fatigue Questionnaire; 8-OH-dG, 8-Hydroxyguanosine ; TnI, Troponin-I; GAA, Guanidine acetic acid

APPENDIX A - Search String

Pubmed

"Biomarkers"[Mesh] OR “biomarker*”[tiab] OR "Vascular Endothelial Growth Factors"[Mesh] OR "Cytokines"[Mesh] OR “Cytokine*”[tiab] OR “EGF Family of Proteins”[Mesh] OR “Endothelial Growth Factors”[Mesh] OR “Brain-Derived Neurotrophic Factor”[Mesh] OR “TGF-beta Superfamily Proteins”[Mesh] OR “Growth Factor*”[tiab] OR Chemokine*[tiab] OR Interleukin*[tiab] OR “Tumor Necrosis Factor*”[tiab] OR “Neurotrophic Factor*”[tiab] OR Neuregulin*[tiab] OR Neurotrophin*[tiab] AND "Fatigue"[Mesh:NoExp] OR “fatigue”[ti] OR “sleep*”[ti] OR “Insomnia”[ti] OR “chemo fog”[ti] OR “brain fog”[ti]) AND “Tumor*”[tiab] OR “Neoplas*”[tiab] OR “Cancer*”[tiab] OR “Oncol*”[tiab])

Embase

"Biomarkers"/exp OR “biomarker*”:ti,ab,kw OR "Vascular Endothelial Growth Factors"/exp OR "Cytokines"/exp OR “Cytokine*”:ti,ab,kw OR “EGF Family of Proteins”/exp OR “Endothelial Growth Factors”/exp OR “Brain-Derived Neurotrophic Factor”/exp OR “TGF-beta Superfamily Proteins”/exp OR “Growth Factor*”:ti,ab,kw OR Chemokine*:ti,ab,kw OR Interleukin*:ti,ab,kw OR “Tumor Necrosis Factor*”:ti,ab,kw OR “Neurotrophic Factor*”:ti,ab,kw OR Neuregulin*:ti,ab,kw OR Neurotrophin*:ti,ab,kw AND 'fatigue'/exp OR ‘fatigue’:ti OR ‘sleep*’:ti OR ‘insomnia’:ti OR ‘chemo fog’:ti OR ‘brain fog’:ti AND ‘neoplas*’:ti,ab,kw OR ‘tumor*’:ti,ab,kw OR ‘cancer’:ti,ab,kw OR ‘Oncol*’:ti,ab,kw

APPENDIX B - Risk of bias figures

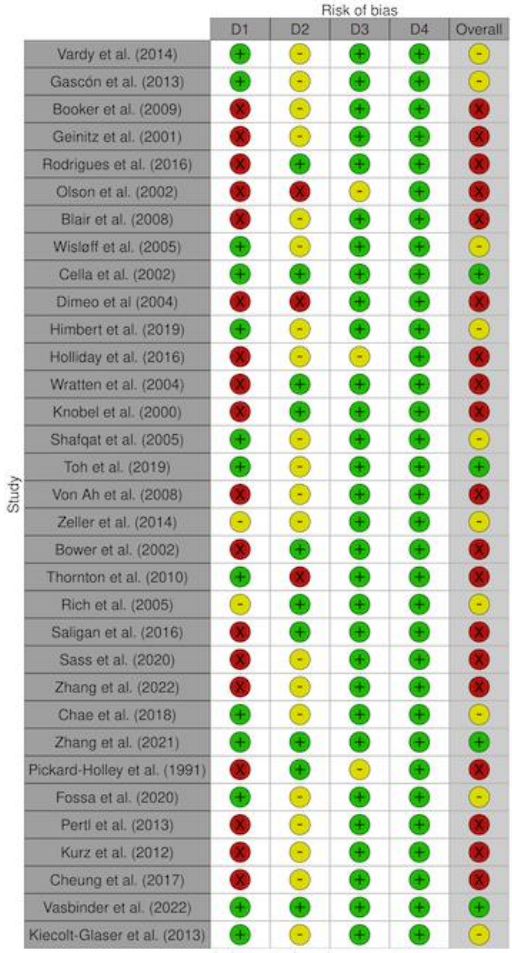

Figure 1: Overview of risk of biases of each article. Figure created with Robvis.

D1: Bias regarding Sample Size, D2: Bias regarding Selection of Participants, D3: Bias regarding Measurement of Outcome, D4: Bias regarding Selection of Reported Results.

Judgement: X High Risk - Some Concerns + Low Risk

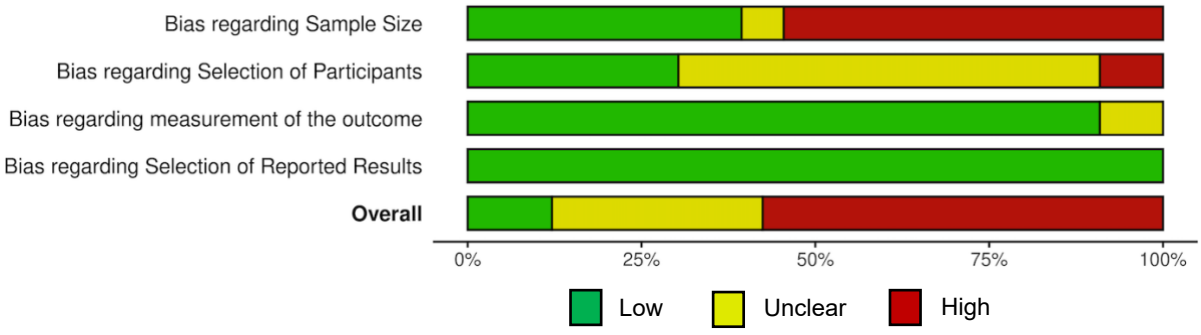

Figure 2: General overview of risk of biases. Figure created with Robvis.

APPENDIX C - Risk of bias and limitations of individual studies

Blood- cells and proteins	
Authors	**Risks of bias and limitations**
Vardy et al. (2014) (38)	- More women than men in HC group - Selection bias (underrepresentation of not fluently English-speaking people) - Race not specified - Multiple significance testing of raw and domain scores
Gascón et al. (2013) (48)	- Heterogenous sample (different types, stages, and therapies) - Treatment bias (treatment of anemia in some patients) - Race not specified
Booker et al. (2009) (59)	- Selection bias (non-probability sampling) - Cross-sectional design - Heterogenous sample (different stages of MM) - Comorbid conditions not taken into account - Race not specified
Geinitz et al. (2001) (49)	- Heterogenous sample (different stages of breast cancer) - Small sample size (n = 41) - Selection bias (underrepresentation of subjects not able to read and understand German) - Race not specified - Treatment bias (some patients also received hormonal therapy or chemotherapy prior to the study)
Rodrigues et al. (2016) (60)	- Evaluation of patients at a single point of their clinical course - Small sample size (n = 51) - Multiple testing
Olson et al. (2002) (50)	- Small sample size (n = 29) - No validated questionnaire - Race not specified - Selection bias (loss of follow-up of n = 11)
Blair et al. (2008) (51)	- Small sample size (n = 40) - Treatment bias (some patients also received radiotherapy before chemotherapy) - Mainly Non-Hispanic white
Wisløff et al. (2005) (52)	- Heterogenous sample (different stages of BC) - Race not specified
Cella et al. (2002) (117)	- Heterogenous sample (different types of solid tumors) - Mainly Caucasian
Dimeo et al (2004) (24)	- Heterogenous sample (different types of hematological malignancies) - Race not specified - Evaluation of the patients' performance status using questionnaires instead of maximal oxygen uptake - Cross-sectional design

Wratten et al. (2004) (46)	- Exclusion of patients with co-morbidities and signs of infection in week preceding assessment - Small sample size (n = 52) - Race not specified

Hormones	
Authors	**Risks of bias and limitations**
Vardy et al. (2014) (38)	- More women than men in HC group - Selection bias (underrepresentation of not fluently English-speaking people) - Race not specified - Multiple significance testing of raw and domain scores.
Knobel et al. (2000) (55)	- Small sample size (n = 33) - Heterogenous sample (different types, stages, and treatment regimens of malignant lymphoma) - Cross-sectional design - Race not specified
Shafqat et al. (2005) (53)	- Heterogenous sample (different types of metastatic cancers) - Cross-sectional design to study fatigue - Selection bias (underrepresentation of subjects not able to read and understand English) - Race not specified
Toh et al. (2019) (40)	- No chemotherapy-naïve cancer controls - Heterogenous sample (different stages of BC) - Selection bias (underrepresentation of subjects not able to read and understand English) - Mainly Chinese
Kiecolt-Glaser et al. (2013) (61)	- Heterogenous sample (different stages of BC) - Race not specified - Selection bias (underrepresentation of subjects not able to read and understand English)
Von Ah et al. (2008) (39)	- Small sample size (n = 44) for the number of predictor variables examined - Limited study design: assessment of CRF after completion of adjuvant therapy. No generalization possible to long-term BC survivors. - Heterogenous sample (stage 0 to stage IIIA BC) - Selection bias (underrepresentation of women who were not able to read and understand English) - Woman only - Mainly Caucasian, married and college education
Zeller et al. (2014) (62)	- Moderate sample size (n = 87) - Heterogenous sample (different types of hematological malignancies) - Selection bias of included CF cases (included survivors were older than those not wanting to participate) - Multiple significance testing (increased risk of type I errors) - Race not specified
Bower et al. (2002) (63)	- Small sample size (n = 40) - Heterogenous sample (stage 0, I, or II BC) - Selection bias (underrepresentation of subjects not able to read and understand English)

	- Mainly white
	- Assessment of cortisol at a single time point, providing limited information about its rhythm
Thornton et al. (2010) (54)	- Single timepoint plasma hormone measurements
	- Selection bias (only women, only recurrent or advanced stage breast cancer)
	- Cross-sectional design
	- Mainly Caucasian
	- Sample size smaller than recommended for the complexity of the model used
Rich et al. (2005) (65)	- Heterogenous sample (different metastasis sites and treatment regimens)
	- Race not specified

Growth Factors and Neurotransmitters

Authors	Risks of bias and limitations
Himbert et al. (2019) (41)	- Biomarker measurements only available at baseline
	- Mainly White
	- Multiple significance testing
	- Other potential confounders (status of anemia, malnutrition, inflammatory bowel disease or diabetes) not available
	- Heterogenous sample (different stages of CRC)
	- Selection bias (underrepresentation of subjects not able to read and understand English or German)
Holliday et al. (2016) (42)	- Small sample size (n = 28)
	- No validated questionnaire
	- Heterogenous sample (different stages of prostate cancer)
	- Mainly Caucasian
Saligan et al. (2016) (116)	- Small sample size (n = 47)
	- Heterogenous sample (different stages of prostate cancer)
	- Mainly Caucasian
Sass et al. (2020) (8)	- Small sample size (n = 40)
	- Plasma samples not collected after fasting or fixed time of the day
	- Samples collected from 2009 – 2014, potential variability due to differences in sample storage time
	- No multiple comparisons correction
	- Isolation method did not discriminate between different EV populations, that may differentially affect CRF
	- Heterogenous sample (different stages of prostate cancer)
	- Mainly white
Fossa et al. (2020) (56)	- Heterogenous sample (different types of lymphomas)
	- Observed metabolic changes not useful for development of diagnostic tests
	- Analysis of serum levels only
	- Cross-sectional design
	- Race not specified
Pertl et al. (2013) (45)	- Blood samples only available for a small number of participants at the follow-up time points

	- Samples collected at variable times of the day - Time gap between assessment of fatigue and blood sampling varied between participants - Only two important confounds (BMI and age) included in the current analyses - Selection bias (underrepresentation of subjects not able to read and understand English) - Heterogenous sample (different stages and treatment regimens of BC) - Race not specified
Kurz et al. (2012) (57)	- Small sample size (n = 50) - Heterogenous sample (different types, different stages, and different treatment regimens of lung cancer) - No direct measure of the enzyme pathways of tryptophan metabolism - Cross-sectional design - Race not specified

Metabolic and Nutritional Markers	
Authors	**Risks of bias and limitations**
Zhang et al. (2022) (66)	- Small sample size (n = 30) - Cross-sectional design - No investigation of the mechanism by which chemotherapeutic drugs such as bortezomib damage liver function and cause guanidinoacetic acid accumulation. - Race not specified
Chae et al. (2018) (43)	- Heterogenous sample (different stages of MM) - No chemotherapy-naïve cancer controls - Selection bias (underrepresentation of subjects who were not able to read and understand English or Chinese) - Mainly Chinese

Markers of Oxidative Stress and Cardiac Damage	
Authors	**Risks of bias and limitations**
Cheung et al. (2017) (58)	- Response bias (proxy- and self-reported measures: the reporting style influenced by response bias, perceived stress of psychological distress of the rater) - No age-matched healthy comparison control group - Mainly white
Vasbinder et al. (2022) (28)	- Heterogenous sample (different stages of BC) - Mainly white - Cross-sectional design - Most participants received radiation in the late 1990s, when radiation doses were higher - Fatigue measured using the SF-36, which was not created specifically for cancer-related fatigue and lacks a multi-dimensional component - Multiple biomarker testing - Wide variation in the timing from BC to post-breast cancer serum collection

Cancer Markers

Authors	Risks of bias and limitations
Zhang et al. (2021) (47)	- Heterogenous sample (different types of cancer) - Selection bias (underrepresentation of subjects not able to read and understand Chinese) - Mainly Chinese
Pickard-Holley et al. (1991) (64)	- Small sample size (n = 24) - Heterogenous sample (different stages of ovarian cancer) - RFS not validated for use in cancer patients - White women only

Abbreviations: HC, healthy controls; CRF, Cancer-Related Fatigue; BC, Breast Cancer; CF, Cancer Fatigue; CRC, colorectal cancer; EV, Extracellular Vesicle; BMI, Body Mass Index; RFS, Rhoten Fatigue Scale; MM, Multiple Myeloma; GAA, Guanidine acetic acid