

PROGNOSTIC VALUE OF PERIPHERAL BLOOD LYMPHOCYTE SUBSETS IN PATIENTS WITH SOLID CANCERS: A RETROSPECTIVE STUDY

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Received 03 Nov 2021, Accepted 26 Nov 2021

<https://doi.org/10.31688/ABMU.2021.56.4.08>

ABSTRACT

Introduction. Lymphocyte subsets are important in regulating immunity and specific killing of tumour cells and appear to be strongly associated with the development of solid tumours. However, their prognostic value in cancer patients remains unclear.

The objective of the study was to determine whether peripheral blood lymphocytes subsets might be prognostic markers for patients with solid cancers.

Material and methods. A databases search was conducted to identify publications from 2010 to 2020 exploring the association of different lymphocyte subsets with overall survival and disease-free survival among cancer patients.

Results. Twenty-nine articles referring to 14 different malignancies were included: 21 evaluated the immune parameters before surgery and 8 after surgery. Sixteen studies reported lymphopenia associated with poor prognosis, other 3 reported increased lymphocytes associated with better prognosis, while 10 studies reported different data: 6 showed correlations between high lymphocyte count and poor prognosis, one study reported low lymphocyte subsets with better prognosis and three reported no impact on prognosis.

RÉSUMÉ

Valeur pronostique des sous-ensembles de lymphocytes du sang périphérique chez les patients atteints de cancer solide: une étude rétrospective

Introduction. Des sous-ensembles de lymphocytes sont importants dans la régulation de l'immunité et la destruction spécifique des cellules tumorales et semblent être fortement associés au développement de tumeurs solides. Cependant, leur pertinence pronostique chez les patients cancéreux reste incertaine.

Le but de l'étude était de déterminer si un sous-ensemble de lymphocytes dans le sang périphérique pouvait constituer des marqueurs pronostiques pour les patients atteints de cancers solides.

Matériel et méthodes. Une recherche dans la base de données de 2010 à 2020 a été menée pour identifier les publications qui explorent l'association de différents sous-ensembles de lymphocytes avec la survie globale et la survie sans maladie chez les patients atteints de cancer.

Résultats. Vingt-neuf articles portant sur 14 tumeurs malignes différentes ont été inclus: 21 paramètres immunitaires évalués avant la chirurgie et 8 après la

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Conclusions. Lymphocyte depletion, before and after surgery, tends to be associated with reduced survival rate. Post-surgery, lymphocyte subsets could have higher predictive value compared to their preoperative levels and regulatory T cells (Tregs) may be a better prognostic indicator of survival.

Keywords: cancer, lymphocytes, overall survival, disease-free survival, prognosis.

List of the abbreviations:

DFS = disease-free survival; OS = overall survival; PFS = progression free survival; RFS = relapse-free survival; NSCLC = non-small cell lung cancer; Tregs = regulatory T cells; CD = cluster of differentiation; NK = natural killer; FoxP3 = forkhead box protein P3

INTRODUCTION

Cancer progression, as well as surgical therapy, can cause stress reactions in patients, which lead to temporary suppression of immune system^{1,2}. In addition, a weak cellular immune function may exert influence on therapeutic efficacy, local recurrence and distant metastases and, consequently, may change into an undesirable prognosis of tumour progression^{3,4}.

The assessment of immune function in cancer patients is mainly based on immune parameters that include T cells (CD3+) specialized in cellular immunity, B cells (CD19+) involved in humoral immunity, and natural killer (NK) cells (CD16+CD56+), that directly kill the tumour cells and virus-infected cells. According to their immunological functions and cell surface markers, CD3+ T cells are mainly subdivided in CD4+ T cells and CD8+ T cells. CD4+ T cells (identified as CD3+ CD4+ T cells) are commonly divided into conventional T helper cells, that play a pivotal role in regulating the immune responses of other immune cells (cytotoxic T cells, B cells and macrophages, and regulatory T cells)^{5,6}, while CD8+ T cells (identified as CD3+ CD8+ T cells) are cytotoxic T cells that kill tumour cells by releasing cytokines, cytotoxic granules or by Fas / FasL interactions⁷. Another major cellular component of immune system, B cells, identified as CD19+ cells, possess distinct functions in both adaptive and innate humoral immune responses. They contribute to antitumour immunity in two ways: they produce antitumour antibodies and present antigens to T cells⁸. NK cells, identified as CD16+CD56+ cells, are the major cytotoxic effectors cells of the innate immune system,

capable of recognizing and destroying tumour cells without prior antigenic exposure, and are involved in coordinating the adaptive immune response^{9,10}.

chirurgie. Seize études ont rapporté une lymphopénie associée à un mauvais pronostic, 3 autres ont rapporté une augmentation des lymphocytes associée à un meilleur pronostic, tandis que 10 études ont rapporté des données différentes: 6 ont montré une corrélation entre un nombre élevé de lymphocytes et un mauvais pronostic, une étude a rapporté de faibles sous-ensembles de lymphocytes avec un meilleur pronostic et trois n'ont rapporté aucun impact sur le pronostic.

Conclusions. La déplétion lymphocytaire avant et après la chirurgie a tendance à être associée à un taux de survie réduit. Après la chirurgie, les sous-ensembles de lymphocytes peuvent avoir une valeur prédictive plus élevée par rapport à leurs niveaux préopératoires, et les cellules T régulatrices (Tregs) peuvent être un meilleur facteur pronostique de survie.

Mots-clés: cancer, lymphocytes, survie globale, survie sans maladie, pronostic.

capable of recognizing and destroying tumour cells without prior antigenic exposure, and are involved in coordinating the adaptive immune response^{9,10}.

Tumours can use several mechanisms to suppress host immunity¹¹; one of the mechanisms refers to regulatory T cells (Tregs) that help tumours evade immune surveillance¹². Tregs are a subpopulation of CD4+ CD25+ T lymphocytes that inhibit antitumour immunity by promoting immune tolerance through direct suppressive functions on T-helper and cytotoxic T cells or by secreting immunosuppressive cytokines such as IL-10 and TGF- β ¹³⁻¹⁵. It is claimed that Tregs express and functionally depend on the transcription factor forkhead box protein P3 (FoxP3)¹⁵.

Although lymphocyte subsets are important in regulating immunity and specific killing of tumour cells and appear to be strongly associated with the development of solid tumours, their prognostic relevance in cancer patients remains unclear. Therefore, the aim of this study was to propose a model that includes the following variables: CD3+, CD4+, CD8+, CD19+, CD16+CD56+, Tregs, the type and severity of the disease and the number of cases and to quantify their effect on overall survival (OS) and disease-free survival (DFS) in different types of cancer. The retrospective review of studies published between 2010-2020 on the prognostic value of peripheral lymphocyte subsets was conducted at diagnosis and/or postoperatively only in patients with solid cancer in different stages of the disease. The study was not extended prior to this period due to the lack of consensus in establishing an essential set of Treg markers.

There are several data that were considered in exploring the impact of peripheral blood lymphocytes

variation on the prognosis of cancer patients before and after surgery: (1) peripheral blood cells can be easily detected and allow real-time monitoring compared to tumour lymphocytes; (2) immune escape of the tumour occurs not only in local immunity but also in systemic immunity; (3) Tregs in peripheral blood are connected to intratumoural Tregs.

MATERIAL AND METHODS

An electronic search from 2010 to 2020 of the following databases: PubMed, SpringerLink and ScienceDirect was performed. The search strategy was to use different keywords referring to the relationship between different peripheral blood lymphocyte subset counts (CD3+ T cells, CD19+ B cells, CD16+CD56+ NK cells, CD4+ T helper cells, CD8+ T suppressor cells, CD4+CD25+FoxP3+ Treg cells) and prognosis, in patients with solid cancer. In addition, to find all potentially relevant studies, the search field was extended to the reference lists of selected articles.

Study selection: In order to reduce the clinical heterogeneity, the following eligibility criteria were utilized: (1) original studies of human subjects with different solid cancers reporting on the prognostic impact of the peripheral blood lymphocytes on OS, and/or DFS or progression-free survival (PFS); (2) only the studies that used the same method of identifying Tregs were selected in this paper; (3) samples collected at diagnosis and/or undergoing surgery treatment; (4) only full text publication; (5) English-language publication. No limitations were applied to the type and severity of cancers, as well as the sex and race of the patients. The exclusion criteria were the following: (1) unrelated to lymphocyte populations established in the selection criteria; (2) case reports, reviews, letters to editors, conference abstracts and (3) insufficient survival information.

Data extraction: The following details were extracted from each study: cancer type, tumour stage, number of patients, immune cell type, prognostic markers used to define immune cell type, prognostic indicator/clinical outcomes, and publication. Survival outcomes annotated included DFS, RFS, PFS and OS.

RESULTS

Studies included and their characteristics

Twenty-nine studies that met the eligibility criteria were included in this review, 21 studies referring to the evaluation of immune parameters before surgery and 8 after surgery.

Table 1 shows the characteristics of the included studies. The publications are reported to be

evenly distributed from 2010 to 2020. Fourteen different solid malignancies (breast, colon, rectum, lung, stomach, kidney, pancreas, liver, esophagus, cervix, nasopharynx, gallbladder, ovary, and bladder) were selected according to the eligibility criteria. The largest number of studies were found for gastrointestinal cancers (colorectal cancer – 6 studies, gastric cancer – 3 studies, esophageal cancer and liver cancer – one study, each one), then for lung cancer (5 studies), breast cancer (4 studies), renal cancer (3 studies) and pancreatic cancer (2 studies). Other types of cancer, such as ovarian, cervical, bladder, gallbladder and nasopharyngeal cancers, are each represented by a single study. The sample size of cancer patients, ranged from 30 (cervical and renal cancer) to 1028 (colorectal cancer). The most common parameter investigated was Tregs (in 8 studies), followed by the absolute lymphocyte count, CD4 and NK (in 6 studies), CD19 and CD4/CD8 (in 5 studies) and CD8 (in one study). Sixteen studies, representing 55.17%, reported lymphopenia associated with poor prognosis. Other 3 studies reported increased lymphocytes, associated with better prognosis, while 10 studies reported different data: 6 of them showed a correlation between high lymphocyte count and poor prognosis; one study reported low lymphocyte subsets with better prognosis and three studies reported no impact on prognosis. The end point was OS in 12 studies, PFS/RFS/DFS in 7 studies and poor prognosis in 9 studies. Three studies showed no impact on survival.

Peripheral blood lymphocyte subsets in patients with solid cancers, before surgery

Twenty-one studies¹⁶⁻³⁶ investigated the prognostic value of peripheral blood lymphocyte subsets in patients with different types of cancer before surgery. The analysis included various solid malignancies such as: colorectal cancer – 5 studies²⁰⁻²⁴, breast cancer – 4 studies¹⁶⁻¹⁹, lung cancer – 4 studies²⁵⁻²⁸, kidney cancer – 3 studies³¹⁻³³, gastric cancer – 2 studies²⁹⁻³⁰ and esophageal³⁴, nasopharyngeal³⁵ and cervical cancer³⁶ – one study. Ten studies performed on patients with different types of cancer have shown that peripheral blood lymphocytes values are independent predictors of OS. Other 5 studies have found associations between the investigated parameters and DFS/RFS or PFS. In contrast, 3 studies did not find associations between the investigated parameters and the clinical outcome.

Peripheral blood lymphocyte subsets in patients with solid cancers, after surgery

Eight studies³⁷⁻⁴⁴ comprising 7 different solid malignancies (gallbladder, pancreatic, liver, colorectal, bladder, non-small cell lung cancer and ovarian

Table 1. Summary of characteristics of the included patients

<i>Cancer type</i>	<i>No of patients</i>	<i>Prognostic marker</i>	<i>Prognostic indicator/clinical outcomes</i>	<i>Ref.</i>
Before surgery				
Clear cell renal carcinoma	424	Low absolute lymphocyte count	Poor OS	[31]
Papillary renal cell carcinoma	192	Overall lymphopenia	Poor OS	[32]
Esophageal cancer	307	Overall lymphopenia	Independent marker of poor prognosis	[34]
Metastatic breast cancer	195	Overall lymphopenia	Independent prognostic factor for PFS and OS	[16]
Rectal cancer and colon cancer	121	High lymphocyte count	Independent favorable prognostic factor (rectal cancer stage I and II); no prognostic significance (rectal cancer stage III and IV and colon cancer)	[20]
Metastatic breast cancer	103	Low absolute CD4+ cells count	Poor OS	[17]
Advanced -NSCLC	86	Low CD3+, CD4+, CD4+/CD8+ and NK cell count	Poor OS	[25]
Advanced- NSCLC	172	High CD4+ cells absolute count	Favorable prognostic factor of PFS	[26]
Metastatic breast cancer	75	High CD4+, CD3+; CD4+/CD8+ ratio, CD56+ and CD19+	Worse survival (high level of CD4+ and CD3+); not prognostic factor (CD4+/CD8+ ratio, CD56+ and CD19+)	[18]
Nasopharyngeal cancer	356	Low CD19+ cells count	Poor PFS and OS	[35]
Gastric cancer	846	Elevated CD19+ cells count	Favorable survival; high DFS	[29]
Colon cancer	224	Low percentage of NK cells CD16+56+	independent prognostic indicator of shorter survival times	[21]
Gastric cancer	122	High NK CD16+56+	Poor OS	[30]
Breast cancer	80	Elevated CD4+/CD8+ ratio	Poor OS	[19]
Early-stage NSCLC	80	Lower CD4+/CD8+ ratio	Better prognosis	[27]
Advanced cervical cancer	30	High CD4/CD8 ratio	Increased risk of having no response to treatment	[36]
Renal cancer	30	High Treg	Worse prognosis	[33]
NSCLC	67	High Treg	Worse clinicopathological conditions and disease recurrence	[28]
Colon cancer	47	High Tregs	No impact on survival	[22]
Colorectal cancer	94 (left colon, 43; rectum, 51)	Low absolute Treg	No impact on survival	[23]
Colorectal cancer and gastric cancer	70 (colon, 37; gastric, 33)	Low Treg	No impact on survival	[24]
After surgery				
Gallbladder cancer	34	Decreased lymphocyte count	Independent factor of DFS	[37]
Pancreatic cancer	390	Lymphopenia	Independent prognostic factor for OS	[38]
Early hepatic cancer	158	CD3+, CD4+, CD8+, NK+, CD19+	Factor of prediction for the recurrence and independent factor of prognostic	[39]
I to III stage colorectal cancer	1028	Low CD4+ cells percentage	independent prognostic factor of RFS	[40]
Pancreatic cancer	56	Low absolute CD19+ cells and high CD16+CD56+cells	independent risk factors of PFS	[41]
Bladder cancer	48	Decreased Treg	Poor prognosis	[42]
NSCLC	49	Decreased Treg	Poor prognosis	[43]
Ovarian cancer	61	Decreased Treg percentages	Poor prognosis	[44]

cancer) explored the association between peripheral lymphocyte subsets and the outcome of patients. Five of these studies³⁷⁻⁴¹ specified association of peripheral blood lymphocytes values with either OS^{38,39} or DFS³⁷, RFS⁴⁰ or PFS⁴¹. Three other studies reported that the investigated parameters are associated with a poor prognosis⁴²⁻⁴⁴.

DISCUSSION

To the best of our knowledge, this is the first review that comprehensively summarizes the prognostic impact of peripheral blood lymphocytes variation on DFS, PFS or OS in patients with various solid malignancies.

Breast cancer in women is the most diagnosed cancer, surpassing lung cancer, and the fifth leading cause of cancer death, according to GLOBOCAN 2020 estimates of cancer incidence and mortality provided by the International Agency for Research on Cancer⁴⁵. Clinical studies revealed that changes in peripheral blood lymphocyte count are closely related to tumour progression and prognosis. In advanced breast cancer, the patients often have lymphopenia and, implicitly, a significantly worse survival than patients with normal lymphocyte counts. Lymphopenia is a powerful predictor of chemotherapy-induced toxicity, in addition to patient's characteristics, disease characteristics and biological parameters¹⁶. Moreover, lymphopenia is associated with an increased risk of febrile neutropenia, thrombocytopenia requiring platelet transfusion, severe anemia requiring red cell transfusion and predisposes the patients to infection, recurrence or to a second malignancy and early death⁴⁶. There are studies reporting that only some of lymphocytes' subsets may be altered in lymphopenic patients. It was found that low levels of absolute CD4⁺ cell counts in peripheral blood at diagnosis are common in patients with advanced breast cancer and are associated with a poor prognosis¹⁷. Other studies showed that, conversely, higher levels of CD4⁺ in HER2-overexpressing metastatic breast cancer patients were significantly correlated with tumour development and a poor prognosis¹⁸. However, the authors state that the results failed to show the statistical significance of CD4⁺ for OS in the multivariate analysis. This may be due to the small sample size or bias caused by unpredictable factors. According to the same authors^{17,18}, CD8⁺, CD19⁺ and CD56⁺ T cell counts have no significant prognostic value for OS. On the other hand, high CD4⁺/CD8⁺ ratio, a parameter useful in monitoring disease progression in cancer patients, has been shown to be significantly correlated with tumour progression and low survival

rate¹⁹. However, the relative role of each lymphocyte subtype is unclear in metastatic breast cancer.

Colorectal cancer is the third cancer in incidence and the second in cancer-related mortality⁴⁵. Despite standard treatment modalities, approximately 40% of patients with colorectal cancer die, indicating a characteristic model of immunodeficiency⁴⁷. As in other malignancies, the prognostic value of peripheral subsets has been suggested as a promising field in colorectal cancer. Studies show that the preoperative increased lymphocytes count is an independent favorable prognostic factor for patients with early-stage rectal cancer. In contrast, in patients with advanced rectal cancer, as well as those with colon cancer, the preoperative lymphocytes counts would not have a prognostic significance²⁰. Among lymphocytes, natural killer (NK) cells could serve as biomarkers for monitoring the immune system, their prognostic value being described in colorectal cancers, where decreases in NK cells number are often observed²¹. Patients with colon cancer who have a significantly lower NK cell counts have shorter survival times than those with higher counts. Other subsets of T lymphocytes involved in the interaction of colorectal tumours and the host immune system and which may affect the immune response against cancer are Tregs lymphocytes. The unambiguous enumeration of Tregs was hampered in the previous years by the inability to directly measure their function and the absence of an exclusive, highly specific marker. The consensus on an essential marker set for Treg enumeration with the currently available markers was established after 2010. Only the studies that used the same method of identifying Tregs, namely CD3⁺CD4⁺CD25⁺FoxP3⁺, were selected in this paper. In colorectal cancer, a heterogeneous disease, with many variations stemming from its primary tumour location, the prognostic role of Treg is controversial²²⁻²⁴. Increases in Treg amounts were observed in some tumour types and sites, or conversely, decreases compared to healthy controls. Tregs were found to be significantly higher in the peripheral blood of patients with advanced colon cancer compared to healthy controls²². This is contradicted by the results obtained by another study, which has found that the absolute number of Tregs in the peripheral blood of patients with left-sided colon cancer has decreased, especially in advanced stages of disease, compared to healthy controls²³. However, the difference between stage I and the other stages did not reach statistical significance, but there was a tendency for a lower Treg counts in stages III and IV than in stage I. This difference was not investigated in a previous study²⁴ due to the small number of patients. All these studies showed that Tregs have no impact on survival. Thus, contrary to the findings observed in other

cancers, no significant relation between the number of Treg cells and prognosis was observed in several studies with colorectal cancer patients. Nevertheless, there are not sufficient data to compare the results of studies.

Lung cancer remains the leading cause of cancer death, with approximately 1.8 million deaths (18%), according to GLOBOCAN 2020 estimates⁴⁵. The immunophenotypic profile on peripheral blood lymphocytes in patients with NSCLC and healthy controls was evaluated in various studies. Furthermore, comparisons between NSCLC patients, with and without metastasis, were also performed. Four studies and 405 eligible NSCLC patients participated in this analysis, showing that immunosuppression is commonly associated in patients with advanced-stage cancers. Thus, a study reported that low levels of absolute CD4+ cell count in the peripheral blood at diagnosis are common in patients with advanced NSCLC²⁵ and are associated with a poor prognosis. Consistently, a high level of absolute CD4+ cells count in patients with advanced NSCLC²⁶ contributed to longer PFS. Prolonged depletion of CD4+ populations appears to lead to prolonged impairment of immune function, limiting the ability of the immune system to eradicate minimal residual neoplastic disease. As well as in colorectal cancer²¹, patients with a significantly lower NK cell count have shorter survival time than those with higher count²⁵. On the other hand, studies showed that low level of CD4+/CD8+ ratio correlates with a better prognosis in the early stages of disease²⁷ and a poor prognosis in advanced disease²⁵. These results are consistent with literature data showing that CD4+/CD8+ ratio decrease can be frequently identified in patients with disseminated disease. The shift in this ratio in cancer patients may be due to a decrease in T-helper and/or an increase in T-suppressor lymphocytes. Therefore, the results of the present study showed that the lymphocyte subsets count in NSCLC with metastasis is significantly lower compared to the lymphocytes in the early-stage NSCLC, supporting the hypothesis that lymphocyte decrease is associated with tumour progression. In addition, since tumour with metastasis is indicative of a poor prognosis, the findings support that lymphopenia is an independent prognostic factor for OS and PFS. Referring to Tregs, it has been observed that an increased number of these lymphocyte subsets accumulate in tumours and the peripheral blood of patients with cancer, and their increased frequency is generally considered to be the marker of a poor prognosis in cancer, presumably due to the Treg-mediated suppression of antitumour immunity. Thus, the significantly increased Tregs are correlated with severe clinicopathological conditions and recurrence²⁸.

Changes in peripheral lymphocyte subpopulations have also been observed in **gastric cancer**. Significantly lower CD3+ and CD8+ percentages and significantly elevated levels of CD4+, CD19+, NK cells, as well as a high CD4+/CD8+ ratio were found in the peripheral blood of 846 gastric cancer patients and 96 control donors. However, it was found that only CD19+ positively correlated with survival²⁹. Increased CD19+ expression appears to improve the humoral immunity, thereby modifying the immune status and prognosis, and further improving the DFS. In contrast, in a recent study³⁰ it was reported that CD19+ B cells in patients were not significantly different from those of controls. Also, CD3+, CD4+, CD8+ T cells, and Tregs in patients were not different from those of controls. Only NK cells proportion in patients was significantly higher than that in healthy donors and is associated with a poor prognosis. This result is in contradiction with the above-mentioned data about colorectal cancer²¹ and NSCLC²⁵. According to the biological function of NK cells, both the direct cytotoxicity and immune modulation effect are involved in NK cell mediated antitumour effects in gastric cancers. It can also be speculated that the sample size (122 patients) may have led to different results. Also, the prognostic values of blood cells based on different cut-off values through different methods were not compared. On the other hand, in a relatively small number of cases (33 patients) a significant decrease in the absolute Treg number was observed compared to healthy controls, but with no impact on patient survival²⁴.

The clinical significance of peripheral blood parameters is a potential prognostic indicator for **renal cancer**. In this review, we found only 3 research groups that evaluated the prognostic value of peripheral blood lymphocyte subsets in patients with renal carcinoma, at diagnosis. Two articles, one on clear cell renal carcinoma (424 patients) and the second on papillary renal cell carcinoma (192 patients) showed lymphopenia, that has been found to predispose the patients to infections, recurrence or to a second malignancy and is associated with a poor PFS and OS^{31,32}. In another article, Treg was evaluated in the peripheral blood of renal cell carcinoma patients based on the most appropriate phenotype CD4+ CD25+ FoxP3+ T cells. It has been shown, as in NSCLC²⁸, that increased Tregs can inhibit antitumour immune responses, leading to tumour development and progression and correlates with a poor prognosis and decreased survival³³.

For other three cancers (**esophageal, nasopharyngeal or cervical**), taking into account the inclusion criteria, only single studies were found in the literature. Thus, in esophageal squamous cell

carcinoma³⁴ it has been found that lymphopenia predisposes patients to infections, recurrence or a second malignancy and is associated with a worse PFS and OS. It has been found that in nasopharyngeal carcinoma³⁵ CD19+ lymphocytes are a predictive factor for survival. Similarly, in advanced cervical cancer patients, the higher CD4+/CD8+ ratio before treatment increases the risk of having no response towards treatment³⁶.

From the published studies, where the assessment of the number of cells was performed only preoperatively, it can be observed that there is no consensus on the extent of changes in different lymphocyte subsets during cancer progression. The use, especially of CD4+ and CD8+, as prognostic markers is unclear, mainly due to the complexity of T helper subsets. Moreover, CD4 + and CD8 + values may be different for various types of cancer. There is also heterogeneity among patients, and among hospitals with respect to technology, which may influence the results. However, it should be noted that most publications show that the absolute values of lymphocyte subsets provide a better indication of the patient's immune status than the percentage values of each lymphocyte subset.

Surgical resection is the most widely used treatment for solid cancers, especially for localized diseases. This may cause stress reactions in patients, leading to immunity suppression, mainly by changing the different lymphocyte subsets count.

Studies reported that the peripheral lymphopenia, especially after surgical resection, has been shown to be an independent predictive and prognostic factor for DFS in *gallbladder cancer*³⁷ and for OS in *pancreatic cancer*³⁸. These studies also suggest that peripheral blood lymphocytes count may have an additive value regarding conventional prognostic factors for death-risk stratification and to predict long-term survival. CD3+, CD4+, CD8+, CD19+ and NK+ markers strongly correlated with survival rate in *hepatocellular carcinoma* before surgery and have a considerable predictive value for malignant characteristics and prognosis. Postoperatively, they are an important predictor of recurrence and an independent prognostic factor³⁹. Also, postoperative peripheral CD4+ T cells percentage is a predictive biomarker for RFS in *colorectal cancer*, this parameter may be useful in identifying patients who will benefit from adjuvant chemotherapy⁴⁰. High absolute counts for peripheral NK cells and low B cell counts may independently predict the progression and were associated with PFS in *pancreatic neuroendocrine tumours* in patients following radical surgical resection⁴¹. In healthy individuals, Treg cells play an important role in the maintenance of self-tolerance. We searched

the literature to see whether surgical treatment in patients affects Tregs level and, implicitly, the survival. From the published data, it was observed that the increase of Treg cells in various cancers, such as *bladder cancer*, *NSCLC* or *ovarian cancer*⁴²⁻⁴⁴ and their decrease after tumour removal at levels almost equal to those of healthy donors correlated with a poor prognosis. It appears that Tregs have a different influence at different stages of cancer development, with an increase in Treg cell number in patients with metastatic cancer, compared to that found in patients with localized disease. The higher proportion of Treg in patients with advanced cancer than in those in early stage suggests the potential role of Treg as a clinical biomarker to indicate a poor prognosis, which may help to stratify patients and adapt their therapy⁴².

Although the peripheral lymphocyte subsets have been shown to be biomarkers of immune status and are related to patient survival in many cancer types, at present, there is a lack of direct evidence for peripheral immune profile in cancer patients. However, clinical practice has shown that the improvement of immune parameters and, implicitly, of immune function, can lead to a favorable prognosis, a better clinical outcome, and a better response to anti-cancer therapy, especially if immunostimulatory treatments are applied. However, from a therapeutic perspective, a simple increase in the absolute lymphocytes count may not be enough to promote clinically significant antitumour responses.

Limitations of the study

This review has some limitations:

- There are differences in patients' age, sex, stage of the disease, histological type, underlying disease, etc., limiting the direct comparison of immune changes in cancers. In addition, these baseline patient characteristics may all have a significant impact on test results.
- The studies focused on different tumours, which are inherently heterogeneous. Heterogeneity was also manifested not only between patients, but also between hospitals in terms of technology (method, antibodies, analysis equipment), which could have affected the results.
- It should be considered that the peripheral blood lymphocytes counts may be affected by concomitant conditions, such as infections, inflammation and medications.
- Due to the substantial heterogeneity in our study, we were unable to perform group statistical analysis based on cancer type, histological grade or clinical stage.
- Only article published in English were included, which could lead to publication bias.

- We did not contact the authors of the included studies to acquire detailed information of patients.

CONCLUSIONS

Several studies investigated the extent to which changes in peripheral lymphocyte subsets correlate with tumour progression and prognosis, in various types of cancer. Although peripheral lymphopenia, identified as pre-existing or induced by surgical therapy in patients with solid tumours, affects survival, there is no consensus on the extent of changes in different lymphocyte subsets during cancer progression or in relationship with response to tumour-specific therapy or survival.

Most of publications refer to the fact that the absolute lymphocytes subset values provide a better indication of the patient's immune status than the percentages of each lymphocyte subsets and should be considered when evaluating the prognosis. However, it can be suggested that a panel of peripheral blood immune markers can complete the patient's immune status and add value to the patient's prognosis, thus being able to direct/redirect the choice of therapy.

Author Contributions:

M.E.P. conceived the original draft preparation. M.E.P., A.B., and L.M.B. were responsible for conception and design of the review. A.M.C., and M.S. were responsible for the data acquisition. M.E.P., A.B., A.M.C., M.S., and L.M.B. were responsible for the collection and assembly of the articles/published data, and their inclusion and interpretation in this review. M.E.P., A.B., A.M.C., M.S., and L.M.B. contributed equally to the present work. All authors contributed to the critical revision of the manuscript for valuable intellectual content. All authors have read and agreed with the final version of the manuscript.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest regarding this article"

"No funding for this study"

Acknowledgements:

None

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