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Lymphocyte count and lymphocyte-to-white blood cells ratio as indicators of survival in specific cancer subtypes

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1. Introduction

Cancer therapy is constantly evolving. Besides chemotherapy, a number of molecular targeted agents, monoclonal antibodies and immune checkpoint inhibitors entered in the therapeutic armamentarium, significantly improving the survival of patients. However, for several solid tumours, especially when diagnosed at an advanced stage, the benefits of treatment in terms of increased survival or quality of life are still, at best, modest, and should be weighed against the potential discomfort caused by medical procedures [1].

The interplay between cell alterations and tumor microenvironment accounts for cancer complexity. Indeed, local invasion and metastasis are more relevant than clonal proliferation and are dependent on multiple mechanisms [2]. In addition the mechanistic locus of metastasis resides within the stroma, and this may explain the shift of research from the cancer cell to the stroma for therapeutic purposes [3].

The role of lymphocytes in cancer immunity is well-established, and immunotherapeutic agents reactivating inhibited CD8 + T-cells have been proven to be effective in various cancer types [4,5]. In certain malignant conditions specific predictive biomarkers playing an essential role in immune function have been identified, namely PD-L1 expression, tumor mutational burden, tumor-infiltrating lymphocytes and microsatellite instability [6]. Many other prognostic and predictive biomarkers emerged to better select patients who will derive the greatest benefit from targeted drugs [7].

Inflammation-related peripheral blood parameters, including the total lymphocyte count (LC), lymphocyte to white blood cells (L/WBC) ratio, neutrophil to lymphocyte (N/L) ratio, or platelet to lymphocyte (P/LC) ratio, have been shown to have prognostic or predictive significance in various cancer types [8–12]. Here we aimed to investigate such easily obtainable parameters in a cohort of patients with advanced solid tumors, to possibly identify in which malignancies they could more efficiently be used to aid clinicians with regard to the decision to discontinue active treatment.

2. Patients and methods

We retrospectively collected the medical records of patients treated at the Oncology Institute of Southern Switzerland, who all died between January 2016 and October 2018 with biopsy-proven advanced solid tumors, including oesophago-gastric, hepatocellular, pancreato-biliary (defined as upper gastrointestinal, uGI), non-small cell lung (NSCLC), small cell lung (SCLC), breast (BC), colorectal (CRC) and prostate (PC) cancer. All patients received at least one line of systemic therapy for metastatic disease.

We extracted data concerning sex, age, primary tumor location, date of diagnosis of metastatic disease, number of treatment lines, cause and date of death. The date of the last prescription of systemic therapy (LPST) with corresponding ECOG performance status and type of anticancer agents were also recorded. A complete blood count with differential white blood cell quantification was extracted both at the time of diagnosis of metastatic disease and at LPST. Blood cell counts were generated using a Siemens ADVIA® 2120i Hematology System device.

Metastatic survival (MS) was defined as the time from diagnosis of metastatic disease to death for any cause. Residual survival (RS) was defined as the time from LPST to death for any cause.

We analyzed the correlation between LC and L/WBC ratio at diagnosis of metastatic disease with MS. We also correlated LC and L/WBC ratio at LPST with RS. We evaluated the association between P/LC ratio at diagnosis of metastatic disease and at LPST with MS and RS, respectively. Finally, we calculated the association between N/L ratio at diagnosis of metastatic disease and at LPST with MS and RS, respectively.

Statistical analysis was performed with the JsStat® software package. The significance threshold was set at $p < 0.05$. The strength of association, measured by Pearson's correlation coefficient r, was interpreted as weak for $0.1 < |r| \leq 0.3$, moderate for $0.3 < |r| \leq 0.5$, and strong for $|r| \geq 0.5$.

Goodness of fit for our linear regression models was measured by the coefficient of determination R^2, expressing the percentage of variability in outcome that can be explained by the parameter under evaluation.

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Table 1
Patients' characteristics %.

Age	
< 70 years	45
≥70 years	55
Gender	
Male	60
Female	40
Site of primary tumor	
Colorectal	14.33
Upper gastrointestinal	24.39
Non small cell lung	29.57
Small cell lung	9.76
Breast	12.8
Prostate	9.15
Number of treatment lines	
1	38.11
2	25
3	15.24
4	8.23
> 5	13.4
ECOG performance status at last prescription of systemic therapy	
0	3.05
1	31.4
2	55.49
3	10.06
Last anticancer agent administered	
Chemotherapy, single agent	28.35
Combination chemotherapy	32.32
Biologic plus chemotherapy	9.15
Biologic, single agent	9.15
Endocrine agent	7.01
Immunotherapy	14.02

This study was approved and carried out in accordance with the recommendations of the local institutional review board.

3. Results

Our study population consisted of 328 patients, distributed as follows: 14.33% CRC, 24.39% uGI, 29.57% NSCLC, 9.76% SCLC, 12.8% BC and 9.15% PC cancer, respectively. Patients' characteristics are summarized in Table 1.

By analyzing the entire cohort of patients, N/L, LC and L/WBC ratio at LPST showed a statistically significant correlation with RS, although the strength of the association was weak (r = −0.13, r = 0.16 and r = 0.24, respectively). However the association was meaningfully stronger in CRC patients for both N/L (r = −0.33), LC (r = 0.47) and L/WBC ratio (r = 0.52) at LPST, and P/LC RATIO also showed a statistically significant association (r = −0.3). A statistically significant correlation for LC and L/WBC ratio also emerged in patients with NSCLC, albeit to a lesser extent (r = 0.2 and r = 0.25, respectively) and for L/WBC ratio in patients with upper GI cancers (r = 0.23). Conversely, no significant difference emerged among the other cancer types.

We also evaluated the relationship between LC, N/L, P/LC and L/WBC ratio at the time of diagnosis of metastatic disease and MS. When we analyzed the entire study population, a statistically significant but weak association was detectable (r = 0.2 for LC; r = −0.18 for N/L; r = −0.16 for P/LC ratio; r = 0.3 for L/WBC ratio). By analyzing separately cancer subtypes, a statistically significant, weak-to-moderate correlation still emerged for CRC (r = 0.3 for LC; r = −0.3 for N/L ratio; r = 0.45 for L/WBC ratio); P/LC ratio did not correlate significantly. A moderate to strong statistically significant association was also observed for SCLC (r = 0.73 for LC; r = −0.47 for N/L; r = −0.44 for P/LC ratio; r = 0.57 for LC/WBC ratio), while no significant correlation was identified for NSCLC. The LC but not L/WBC ratio was significantly associated with MS in BC (r = 0.39).

Table 2
Laboratory parameters and survival by cancer: Pearson's correlation coefficient r and goodness of fit (R^2).

	MS by LC at DMD	MS by L/WBC at DMD	MS by N/L at DMD	RS by LC at LPST	RS by L/WBC at LPST	RS by N/L at LPST	MS by P/LC at DMD	RS by P/LC at LPST
CRC	**0.3** (0.09)	**0.45** (0.21)	**−0.3** (0,09)	**0.47** (0.22)	**0.52** (0.27)	**−0.33** (0.11)	−0.23 (0.05)	**−0.3** (0.09)
Upper GI	−0.02 (0)	**0.25** (0.06)	−0.16 (0.02)	0.1 (0.01)	**0.23** (0.05)	−0.03 (0)	−0.12 (0.01)	−0.02 (0)
NSCLC	0.2 (0.04)	**0.25** (0.06)	−0.17 (0.03)	**0.2** (0.04)	**0.25** (0.06)	−0.14 (0.02)	**−0.22** (0.05)	−0.03 (0)
SCLC	**0.73** (0.53)	**0.57** (0.32)	**−0.47** (0.22)	0.07 (0)	0.04 (0)	−0.12 (0)	**−0.44** (0.19)	0.18 (0.03)
Breast	**0.42** (0.17)	0.25 (0.06)	−0.29 (0.08)	−0.04 (0)	0.03 (0)	−0.1 (0.01)	−0.25 (0.06)	−0.1 (0.01)
Prostate	0.02 (0)	0.05 (0)	0 (0)	0.21 (0.04)	0.11 (0.01)	−0.22 (0.05)	−0.09 (0.01)	0.0 (0)
All	**0.2** (0.04)	**0.3** (0.09)	**−0.18** (0.03)	**0.16** (0.03)	**0.24** (0.06)	**−0.13** (0.02)	**−0.16** (0.03)	−0.06 (0)

DMD = diagnosis of metastatic disease; LPST = last prescription of systemic therapy; LC = lymphocyte count; L/WBC = lymphocyte to white blood cell ratio; N/L = neutrophil to lymphocyte ratio; P/LC = platelet to lymphocyte ratio; MS = survival from diagnosis of metastatic disease to death; RS = survival from LPST to death.
Correlation: r–0,30 = weak; r–0,50 = moderate; r–0,70 = strong.
Values in bold are statistically significant (p < 0.05).

The N/L ratio appeared to perform similarly to LC and L/WBC ratio, but overall it correlated slightly less well with patient survival.

In the entire group, the P/LC ratio at diagnosis of metastatic disease showed an overall weak but statistically significant inverse correlation with MS (r = −0.16); no relationship emerged between P/LC ratio at LPST with RS. By analyzing separately cancer subtypes, an inverse correlation between P/LC ratio at diagnosis of metastatic disease and MS emerged in SCLC (r = −0.44) and NSCLC (r = −0.22).

Table 2 summarizes full data according to inflammation-related peripheral blood parameters and survival by cancer subtype.

Figs. 1 to 3 show scatter plots and relative regression curves for the most relevant pairs of variables as described above.

4. Discussion

Systemic inflammation is a well established factor of poor outcome in cancer and also plays a role in cancer-related symptoms such as weight loss, lack of appetite, fatigue, physical deconditioning, depression and pain. Dysregulation of inflammatory response can be easily evaluated through laboratory parameters such as LC, L/WBC ratio, N/L ratio, P/LC ratio, C-reactive protein and prognostic scores such as the Glasgow Prognostic Score [13,14]. Such parameters reflect the burden of systemic inflammation, but they give no information concerning the specific interactions that occur between immune cells, cancer cells and surrounding microenvironment.

Based on the hypothesis that laboratory parameters related to inflammation and immunity might be modified by the microenvironment specific to the tumor subtype and knowing that systemic therapy might influence the dynamics of immune balance over time, here we aimed to investigate changes of the inflammatory-related blood parameters during the course of disease in patients with advanced solid malignancies and correlate them with survival from diagnosis of metastatic disease and from LPST.

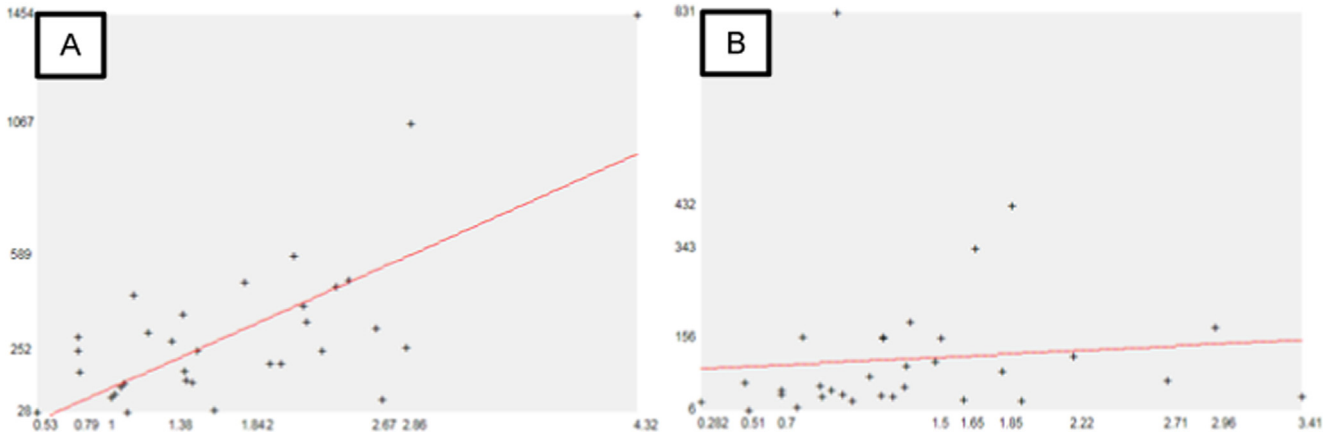

Fig. 1. SCLC: Lymphocyte count and survival (days). A: At diagnosis of metastatic disease: statistically significant correlation: r = 0.73, p < 0.05, R^2 = 0.53. B: At last prescription of systemic therapy: not significant correlation: r = 0.07, p = 0.703, R^2 = 0.

The main finding in our study is that the correlation between LC or L/WBC ratio and RS after LPST appears to be driven by CRC and lung cancer. Interestingly, the correlation between such inflammation-related blood parameters at diagnosis of metastatic disease and MS was stronger in both CRC and SCLC than in NSCLC patients, and a greater percentage of the variability in outcome could be attributed to these parameters according to goodness of fit testing. For instance, LC at diagnosis of metastatic disease explained as much as 53% of the variability in MS in SCLC patients, while it only accounted for a not significant 4% in NSCLC patients; similarly, L/WBC ratio explains 32% of the variation in MS in SCLC compared to 6% in NSCLC. The efficacy of immune checkpoint inhibitors in the treatment of NSCLC may account for this difference. Indeed, anti-PD1 and anti-PDL1 agents, nowadays prescribed in early lines of therapy in NSCLC, might be able to correct a deficient immunological status, therefore confounding baseline predictions.

The blood parameters at diagnosis of metastatic disease performed better in SCLC, a malignancy with only few effective treatment lines, in which one could speculate a considerably predominant role of the immunological status in determining prognosis. The finding that the prognostic value of these parameters was not retained at LPST is probably due the intrinsic clinical aggressiveness of SCLC.

Interestingly, SCLC histology also exhibited the strongest correlation among all cancer subtypes between P/L ratio and N/L ratio at diagnosis of metastatic disease and MS, but again the correlation was lost at LPST.

To prevent the occurrence of cancer and the development of metastasis, the immune system acts by balancing immune activation and tolerance. Some aspects of this regulation are organ- and tissue-specific, as shown in immune-privileged organs such as the central nervous system, testis, pregnant uterus and eye [15,16]. Moreover, organs steadily exposed to external pathogens display the ability to induce an effective immune response while at the same time preventing immune hyperactivation that could result in tissue damage [17]. Changes in immune competence also occur over time, mediated by sensitization and exhaustion that follows protracted antigenic stimuli [18].

Besides the skin, the GI tract and lung are the main organs in direct contact with the external environment and host a variety of non-self antigens influencing the immune balance [19].

If in NSCLC the advent of immune-checkpoint inhibitors has changed the paradigm of treatment, these drugs do not exhibit efficacy in CRC, except for particular conditions (namely the presence of microsatellite instability). Both the GI tract and, to a lesser extent, the lung are colonized by a microbiome that may undergo changes under various pathological conditions [20]. The mechanisms of immune tolerance in the GI tract have not been completely elucidated, and anatomical and physiological differences between different digestive organs may account for the immune response variability [21].

The main limitations of our study are the retrospective character and the small size of the patient cohort, that makes it more challenging to identify outliers that may influence regression analyses. In addition, even though statistically significant correlations were identified between the inflammatory blood parameters and survival, testing for

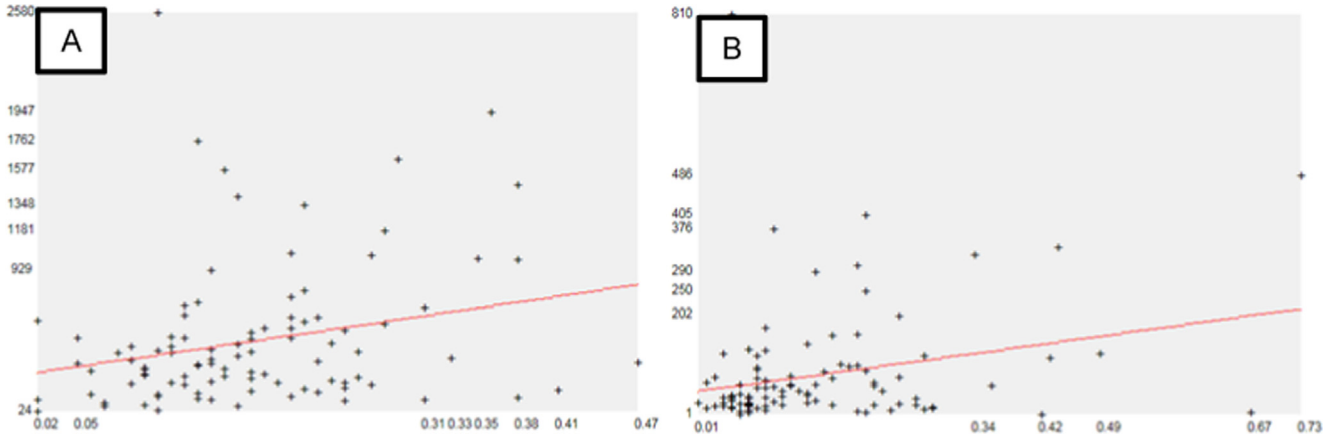

Fig. 2. NSCLC: Lymphocyte count to white blood cell ratio and survival (days). A: At diagnosis of metastatic disease: statistically significant correlation: r = 0.25, p < 0.05, R^2 = 0.06. B: At last prescription of systemic therapy: statistically significant correlation: r = 0.25, p < 0.05, R^2 = 0.06.

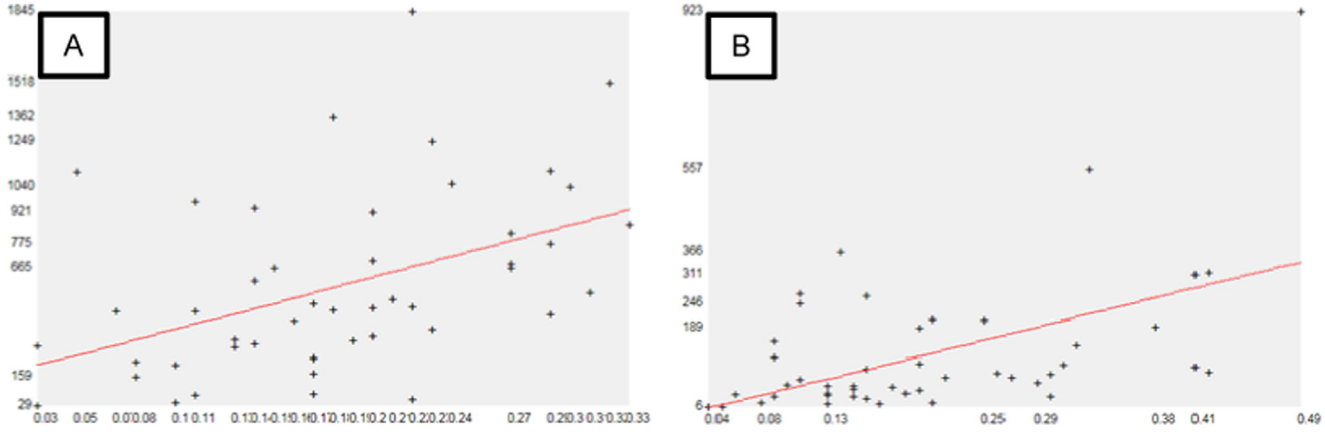

Fig. 3. CRC: Lymphocyte count to white blood cell ratio and survival (days). A: At diagnosis of metastatic disease: statistically significant correlation: $r = 0.45$, $p < 0.05$, $R^2 = 0.21$. B: At last prescription of systemic therapy: statistically significant correlation: $r = 0.52$, $p < 0.05$, $R^2 = 0.27$.

goodness of fit showed that only a limited percentage in outcome variability could be attributed to the parameters themselves, particularly in NSCLC. The use of glucocorticoids, either as part of the antiemetic prophylaxis or for the management of toxicities or concurrent conditions, must also be acknowledged as a possible confounding factor, due to their ability to induce neutrophilia and lymphocytopenia and capability to interfere with the immune response [22].

To conclude, in our study LC and L/WBC ratio are significantly associated with RS after LPST of an active anticancer treatment in CRC and NSCLC patients, and they might contribute with other factors in the difficult decision to stop active treatments near the end of life. The inflammation-related peripheral blood parameters also appear to be significantly associated with MS in CRC and SCLC, to a lesser extent in NSCLC and uGI patients.

Many variables contribute to the decision to continue active anticancer treatments, and our preliminary findings might form a platform for well designed, prospective clinical trials.

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