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ORIGINAL ARTICLE



# Serum parameters as prognostic biomarkers in a real world cancer patient population treated with anti PD-1/PD-L1 therapy

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**ABSTRACT**

**Background:** Immune checkpoint inhibitors (ICI) are regarded as a standard of care in multiple malignancies. We hypothesized that serum parameters are of prognostic value in ICI treated patients suffering from solid tumours.

**Methods:** Data from 114 patients treated with ICIs for solid malignancies from 2015 to 2019 at the Medical University of Vienna were collected retrospectively.

Data included baseline characteristics, cancer type, serum parameters such as lactate dehydrogenase (LDH), C-reactive protein (CRP), albumin (Alb) and lymphocyte counts as well as overall survival (OS) and progression free survival. Additionally, the Gustave Roussy Immune Score (GRIm score) and the Glasgow prognostic score (GPS) were calculated. Cox regression models including time-dependent effects and strata for tumour type were used. Prognostic factors were pre-selected using a relaxed LASSO approach.

**Results:** The majority of patients were male (64.9%). The most common cancer types were non-small cell lung cancer (30.7%) and renal cell carcinoma (21.9%). Increased LDH and CRP were associated with poor 6-month OS (Hazard ratios (HR)=1.16 and 1.06 per 20% LDH/CRP increase; 95% CI 1.07–1.26 and 95% CI 1.03–1.09, respectively; $p < .001$). Both GRIm Score and GPS had a significant influence on OS (GRIm: HR = 2.84, 95% CI 1.72–4.69; $p < .001$ for high vs. low; GPS HR 3.57, 95% CI 1.76–7.25; $p < .001$ for poor vs. good). The proportion of explained variation (PEV) of our full multivariable model was significantly higher compared to the GRIm and GPS (PEV = 29.5% vs. 14.8% and 14.65%). When grouped into quartiles according to the individual 8-weeks change, both increased LDH and CRP correlated with poor OS (LDH ($p$=.001) and CRP ($p < .001$)).

**Conclusion:** The results of this analysis suggest that serum parameters might have prognostic value for the outcome of cancer patients treated with ICI, regardless of the tumour type.

**KEY MESSAGES**

- In this retrospective analysis, 114 patients with solid tumours were included. The results of this analysis point out that pre-treatment LDH, CRP and albumin levels are strongly prognostic for a poor 6-month OS.
- In addition to that, a high GRIm-score and poor GPS were associated with a worse OS (GRIm: HR = 2.84, 95% CI 1.72–4.69; $p < .001$ for high vs. low; GPS HR = 3.57, 95% CI 1.76–7.25; $p < .001$ for poor vs. good).
- Pre-treatment serum parameters might have prognostic value for the outcome of cancer patients treated with ICI, regardless of the tumour type.

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## 1. Introduction

Immune checkpoint inhibitors (ICI) targeting the programmed cell death protein ligand 1 (PD-1/PDL-1) axis have entered the clinical routine during the last couple of years. ICI therapy is regarded as the standard of care for a wide range of malignancies such as metastatic non-small cell lung cancer (NSCLC), head and neck squamous cell carcinoma (HNSCC), renal cell carcinoma (RCC). While ICI therapy was shown to enable unprecedented response duration and even long-term

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survival in patients with stage IV disease, ICI are pricey and economic criteria have to be taken into account as well: In patients suffering from RCC or HNSCC, ICI were less likely to be cost-effective than in patients with melanoma or lung cancer as reported recently [1]. From a clinical perspective, however, not all patients benefit from immunotherapy: A recent analysis of 262 patients suffering from 19 different malignancies demonstrated an objective response rate of 29% across all tumour types and a long-term survivor rate (i.e. longer than 2 years) of 11.8% [2]. Response to single agent ICI was shown to vary considerably between highly sensitive tumours such as Hodgkin's lymphoma and resistant tumours such as microsatellite stable colorectal cancer [3,4].

In the real-life setting, immunotherapy is frequently given in patient populations, which were initially excluded from the respective clinical trials, due to its beneficial side effect profile. In HNSCC, for instance, it was demonstrated that the prognosis of patients with an ECOG PS $\geq$2 or in the platinum resistant setting is worse compared to platinum sensitive patients with a better ECOG PS [5]. In this context, it seems evident that the identification prognostic biomarkers for cancer patients treated with ICI is highly desirable both from an economic and from a clinical perspective.

Peripheral blood is an easily accessible source for potential biomarkers and serum parameters such as lactate dehydrogenase (LDH) are usually measured for routine purposes. Therefore, various scores such as the Gustave Roussy Immune Score (GRIm) and the Glasgow prognostic score (GPS) were established as prognostic tools in patients undergoing ICI therapy [6–9]. The GRIm score is based on the three independent biomarkers albumin (Alb), LDH and neutrophil-to-lymphocyte ratio, while the GPS incorporates the inflammation marker C-reactive protein (CRP) and Alb to predict outcome of cancer patients [6–8,10,11]. However, the majority of these studies investigated the GRIm score, GPS or selected serum parameters in a single tumour type.

In this retrospective analysis we investigated both the potential prognostic value of multiple serum parameters and prognostic scores (such as GRIm or GPS) in a heterogeneous patient population treated with ICI and suffering from a wide range of solid tumours.

## 2. Methods

### 2.1. Study population

This study was designed as retrospective analysis of observational data. The data of patients who started single-agent pembrolizumab or nivolumab between 01 January 2015 and 31 November 2016 were included and followed up until 01 July 2019, which was defined as data cut-off date for this analysis. This work was approved by the ethics committee of the Medical University of Vienna (2132/2016). Reporting of the study conforms to broad EQUATOR guidelines [12].

#### 2.1.1. Inclusions criteria

Stage IV pembrolizumab or nivolumab treated solid tumours except melanoma patients.

Serum LDH, CRP, Alb levels, white blood cell (WBC) count $<$1 week before start of treatment known.

#### 2.1.2. Exclusions criteria

LDH, CRP, Alb levels prior to treatment unknown.

### 2.2. Data collection and endpoint assessment

LDH, CRP, Alb, WBC, NLR, lymphocyte count (LC) and body mass index (BMI) were collected $<$1 week prior to therapy start and after 6–8 weeks after therapy start. The data was retrospectively extracted from the medical files. The GRIm score and GPS were calculated as described previously [8,9]. As for GPS, we used 0.5 mg/dl (instead of 0.1 mg/dl) as the upper limit of normal for CRP as outlined above.

For graphical representation of NLR, patients were divided into three groups (group 1: NLR $<$ 2.5, group 2: NLR $=$ 2.5–6 and group 3: NLR $>$ 6) [8].

Differences in LDH, Alb and CRP levels prior treatment and 6–8 weeks after therapy start were calculated. Patients were classified according to quartiles of this difference. Patients in the lower quartile groups had the highest increase in LDH, Alb or CRP level, whereas patients in the higher quartile groups had a lower increase or decrease of the respective parameter. Overall survival (OS; time from the first ICI dose until death from any cause) and progression free survival (PFS; time from the first ICI dose until disease progression or death from any cause) were chosen as primary endpoints.

Raw data were generated at the Medical University of Vienna. Derived data supporting the findings of this study are available from the corresponding author TF on request.

### 2.3. Statistical analysis

Categorical variables are presented as counts and percentages. Due to asymmetrical distributions,

continuous variables are presented as median and quartiles or range. For selected serum parameters as well as for proposed classification scores, Kaplan-Meier curves for OS and PFS are presented according to established cut-off values. Due to time-dependent effects, no log-rank test results are provided. Instead, *p*-values are derived from the univariable Cox regression models detailed below. A relaxed LASSO approach was used to select prognostic factors out of a pre-selected list (LDH, CRP, Alb, LC, BMI, WBC, neutrophil granulocytes (NG), monocytes (MO), basophile granulocytes). This was done separately for OS and PFS and did not include potential time-dependent effects. Univariable Cox regression models were then applied to each of the selected variables to test cubic and quadratic terms and to check the proportional hazards assumption (by testing interactions with time or log of time and selecting the one with lower AIC). Independent variables were log-transformed with basis 1.2 if necessary, to avoid overly influential extreme observations. Hazard ratios (HR) for such variables consequently quantify the effect of a 20% increase. For the three-category GPS score, an overall *p*-value from a 2 degrees-of-freedom test is reported while HRs with 95% confidence intervals (CI) are given with respect to category "good" as a reference.

All Cox regression models include a stratification for four tumour types (NSCLC, HNSCC, RCC, other) to account for potential heterogeneity in the baseline hazard function and to test for effects that are uniform across malignancies.

For independent variables exhibiting a significant time-dependence of their effect, hazard ratios are presented at 6, 12 and 24 months with 95% CI. Overall, *p*-values from a 2 degrees-of-freedom test for the main effect and the interaction with (log of) time are given. For the remaining variables, a single hazard ratio with 95% CI and the corresponding *p*-value is provided. A multivariable model includes all variables selected by the relaxed LASSO approach and time-dependent effects if necessary. Results are presented in the same way as for the univariable models.

Due to time-dependent effects, the proportion of explained variation (PEV [13]) is reported only for short-term prediction by censoring all observations at 6 months. In order to investigate the potential effect of a change in serum parameters on OS, a landmark approach was used: All patients died or censored before 8 weeks after therapy start were deleted, and the time starting from 8 weeks after start of ICI therapy was investigated using Cox regression models with the difference of the serum parameter between baseline and 8 weeks as independent variable. Due to extreme outliers in these differences, results are presented for quartile groups (i.e. the 8-weeks differences were categorized into four groups according to observed quartiles). The *p*-values are reported which cover the effect for all quartile groups as well as time-dependent terms.

R package "penalized" was used for employing the LASSO approach. All remaining analyses were done using SAS 9.4 (SAS Inc., 2016). Two-sided *p*-values below .05 are regarded to indicate statistical significance.

## 3. Results

One hundred and fourteen patients met the inclusion criteria and were eligible for this analysis. The most common cancer types were NSCLC (30.7%) and RCC (21.9%). The majority of patients (62.3%) received >1 prior therapy lines. ICI therapy was either nivolumab (48.1%) or pembrolizumab (51.8%), mean number of ICI therapy cycles was 6 (range 1–36). In addition to that, smoking status was collected: 19 (21.1%) were current smokers, 35 (38.9%) were former smokers and 36 (40%) were never smokers. From 24 patients, smoking status was not available. Demographic data are shown in Table 1.

In order to account for this heterogeneous patient population and in attempt to identify prognostic

**Table 1.** Demographic data.

| | *n* (%) |
|---|---|
| Patients | |
| Total | 114 |
| Female | 40 (35.1) |
| Male | 74 (64.9) |
| Median age (years) | 60 (range 22–88) |
| Diagnosis | |
| Lung cancer | 35 (30.7) |
| Renal cancer | 25 (21.9) |
| Head and neck cancer | 11 (9.6) |
| Breast cancer | 5 (4.4) |
| Colorectal cancer | 5 (4.4) |
| Sarcoma | 10 (8.8) |
| Urothelial cancer | 12 (10.6) |
| Others | 11 (9.6) |
| Prior therapy lines | |
| 0 | 7 (6.4) |
| 1 | 33 (30.0) |
| 2 | 26 (23.6) |
| ≥3 | 44 (40.0) |
| Missing | 4 |
| Therapy | |
| Nivolumab | 55 (48.2) |
| Pembrolizumab | 59 (51.8) |
| Mean cycle number (range) | 6 (1 – 36) |
| Smoking status | |
| Current | 19 (21.1) |
| Former | 35 (38.9) |
| Never | 36 (40.0) |
| Missing | 24 |

markers beyond tumour biology, we employed stratification for four tumour types as outlined above. Heterogeneity of included tumour types and the influence of the distinct tumour biology on OS is depicted in Figure 1.

### 3.1. Association of pre-treatment serum parameters with outcome

Thirty-one (27%) and 93 (82%) patients presented with an increased LDH or CRP. Increased LDH and CRP were associated with both a shorter median OS (mOS) (3.7 months vs. 13.6 months, $p < .001$; 7.9 months vs. 23.3 months, $p < .001$) and PFS (2 months vs. 5 months, $p < .001$; 3.0 months vs. 7.0 months, $p < .001$; Figure 2(A–D); Table 2).

An inverse association was found regarding Alb or LC: decreased Alb (33% of patients) and LC counts), (27% of patients), had a negative impact on OS (15.6 months vs. 3.3 months, $p < .001$ and 16.4 months vs. 11.2 months, $p = .001$). PFS was not associated with Alb or LC count (Figure 2(E–H); Table 2).

To further confirm the effect of these serum parameters based on their continuous values we present results from Cox regression models for a 20% increase of baseline LDH, CRP and LC) or an increase by 10 g/l Alb on OS and PFS.

In the univariable analysis using stratification by tumour type, both LDH and CRP were significantly associated with OS ($p < .001$ for both) but exhibited a time-dependent effect. While OS was significantly worse in patients with LDH or CRP increase at 6 months (for 20% increase: LDH HR = 1.16, 95% CI 1.06–1.26; CRP HR = 1.06, 95% CI 1.03–1.09), the effect

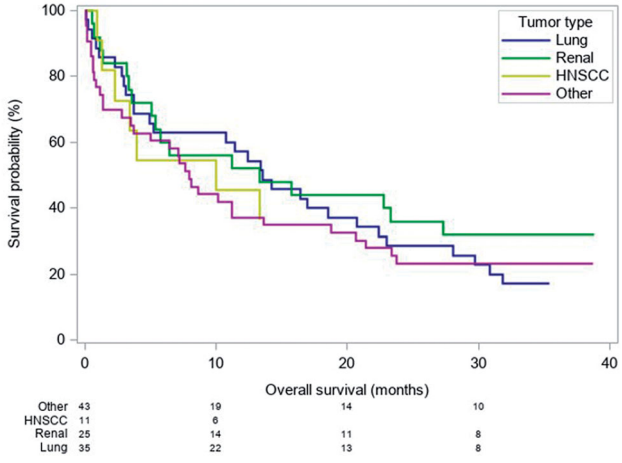


Figure 1. Comparison of OS by tumour entity. Heterogenity of included cancer subtype and influence on OS. HNSCC: Head and neck squamous cell carcinoma; Lung: lung cancer; Renal: renal cell carcinoma.

Table 2. Median OS and 25–75 percentile according to serum LDH, CRP, Alb and LC at therapy start.

| | Median OS | 25%–75% | Median PFS | 25%–75% |
|---|---|---|---|---|
| LDH ≤ 250 U/l | 13.6 | 3.9–36.5 | 5.0 | 2.0–13.0 |
| LDH>250 U/l | 3.7 | 0.7–13.6 | 2.0 | 0.5–4.0 |
| CRP ≤ 0.5 mg/dl | 23.3 | 8.6–n.r. | 7.0 | 3.0–22.0 |
| CRP>0.5 mg/dl | 7.9 | 1.4–23.0 | 3.0 | 1.0–9.0 |
| Alb ≥ 35 g/l | 15.6 | 5.1–37.7 | 5.0 | 2.0–13.0 |
| Alb < 35 g/l | 3.3 | 0.6–13.3 | 1.5 | 0.5–7.0 |
| LC ≥ 1 G/l | 16.4 | 2.9–36.5 | 4.0 | 1.0–13.0 |
| LC < 1 G/l | 11.2 | 3.9–20.7 | 4.0 | 2.0–9.0 |

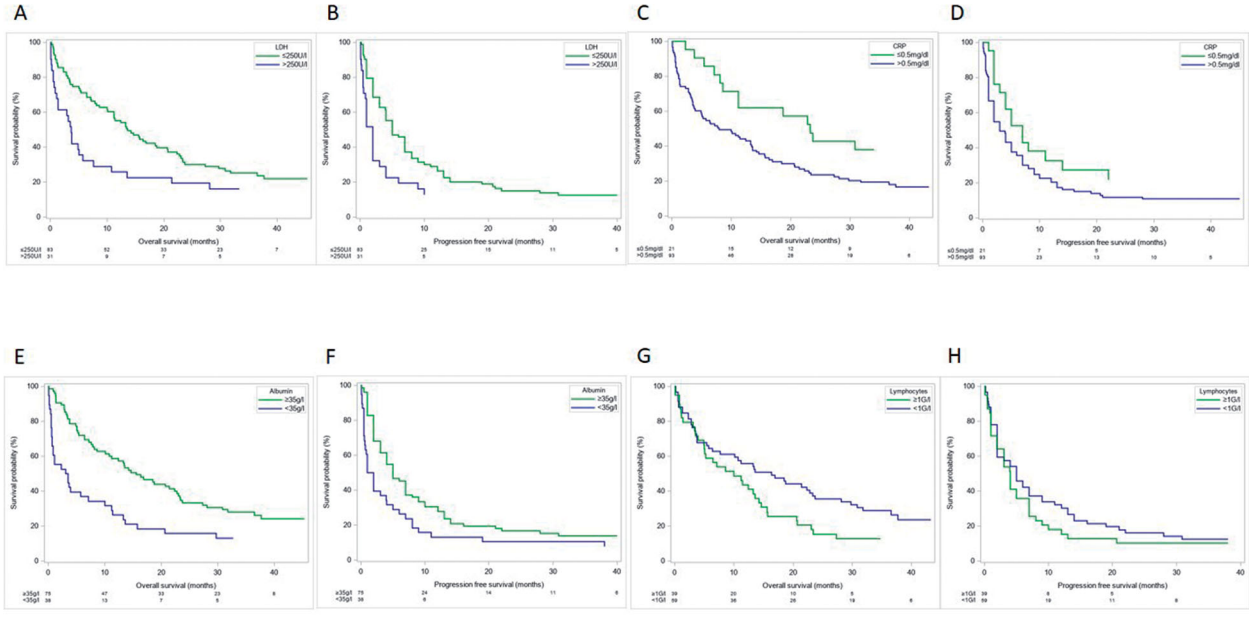


Figure 2. Pre-treatment serum levels and their unadjusted association with OS and PFS. (A) OS curve for LDH, (B) PFS for LDH, (C) OS curve for CRP, (D) PFS for CRP levels, (E) OS curve for serum Alb, (F) PFS curve for serum Alb, (G) OS curve for LC and (H) PFS curve for LC.

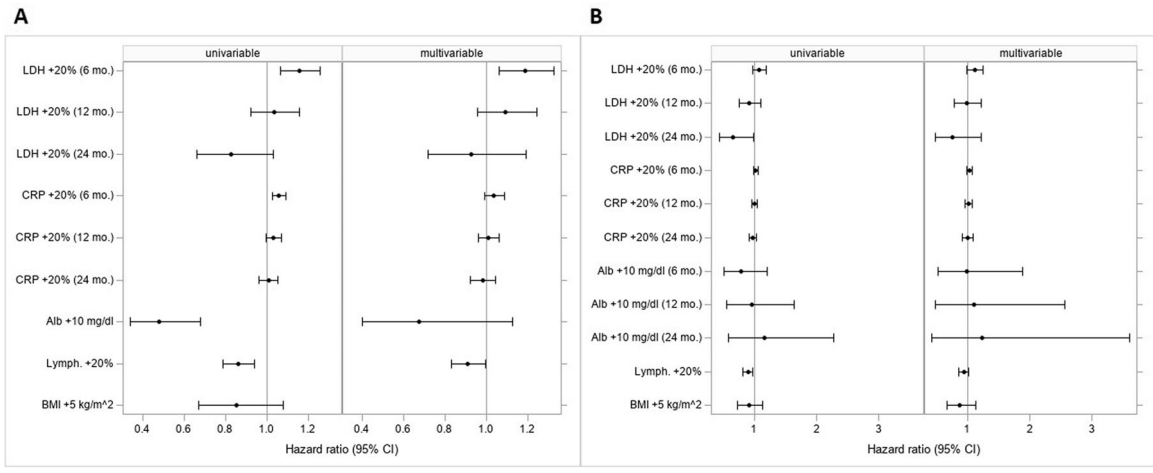


**Figure 3.** Univariable and multivariable analysis of the effect of baseline covariable value on OS (A) and PFS (B). Hazard ratios (with 95% confidence intervals) quantify the effect resulting from a change in the respective baseline covariable value. Effects of covariables entering the model after log-transformation refer to 20% increases, those of other variables to increases by the given units. Time-dependent effects are reported at 6, 12 and 24 months. Confidence intervals not including 1 indicate statistical significance. Both, univariable and multivariable models are stratified for tumour type.

**Table 3.** Median OS and HR of predefined scores.

| GPS | mOS | 25%–75% | HR | 95% CI | mPFS | 25%–75% | HR | 95% CI |
|---|---|---|---|---|---|---|---|---|
| Good | 23.3 | 8.6–n.r. | 1 | | 7 | 2–30.8 | | |
| Intermediate | 14.2 | 5.0–37.7 | 1.38 | 0.7–2.75 | 5 | 2–12 | 1.41 | 0.76–2.64 |
| Bad | 2.3 | 0.6–11.4 | 3.57 | 1.76–7.25 | 1 | 0.5–6 | 2.70 | 1.39–5.27 |
| GRIm | | | | | | | | |
| 0–1 Low | 14.7 | 5.3–37.7 | 1 | | 5 | 2–13 | | |
| 2–3 High | 1.1 | 0.6–7.1 | 2.84 | 1.72–4.69 | 1 | 0.5–4 | 2.39 | 1.48–3.87 |

mOS in month with the respective 25 + 75 percentile.

subsided within 12 months (LDH: HR = 1.03, 95% CI 0.93–1.16; CRP: HR = 1.03, 95% CI 1.00–1.07). However, overall *p*-values showed a statistically significant association of increased LDH and CRP levels with PFS ($p < .001$). Particularly for a time period of 24 months increased LDH was associated with better PFS (HR 0.66, 95% CI 0.44–0.99), although, this effect was diminished in the multivariable model (Figure 3).

Conversely, an increase of Alb or LC was associated with a significantly improved OS in the univariable models (10 g/dl increase of Alb: HR = 0.48, 95% CI 0.34–0.68; $p < .001$; 20% increase of LC: HR = 0.86, 95% CI 0.79–0.94; $p = .001$). Similarly, an increase of LC was associated with significantly improved PFS (HR = 0.90, 95% CI 0.82–0.98; $p = .013$; Figure 3). There was no statistically significant association between BMI and OS or PFS.

In the multivariable analysis, the relaxed LASSO approach revealed LDH, CRP, Alb and LC as prognostic factors for OS (while BMI, WBC, NG, MO, BG were not selected; BMI was selected for PFS only). LDH, CRP and LC remained statistically significant predictors of OS (overall, $p = .002$, .013 and .038, respectively). For LDH, the point estimate at 6 months proved to be statistically significant (HR = 1.19, 95% CI 1.06–1.33).

An increase in LC was significantly associated with better OS (no time-dependent effect; HR = 0.91, 95% CI 0.83–0.99; $p = .038$; Figure 3).

### 3.2. Gps, NLR and GRIm score

The GPS, a validated tool to stratify prognostic groups in cancer patients, was calculated for 113 patients. Subsequently, patients were grouped accordingly (good: 17 (15%), intermediate: 61 (54%) and poor: 35 (31%)). The mOS and PFS differed markedly between the groups ((good: 23.3 months, intermediate: 14.2 months, poor: 2.3 months; overall $p < .001$) and good: 7 months, intermediate: 5 months, poor: 1 month, overall $p = .004$)) (Table 3). The "poor" cohort had a significantly shorter OS and PFS compared to the "good" group (HR = 3.57, 95% CI 1.76–7.23 and HR = 2.70, 95% CI 1.39–5.27; Figure 4(A,B); Table 3).

NLR was available for 98 patients. The mOS and PFS were significantly shorter the higher the NLR (mOS group 1: not reached, group 2: 13.8 months,

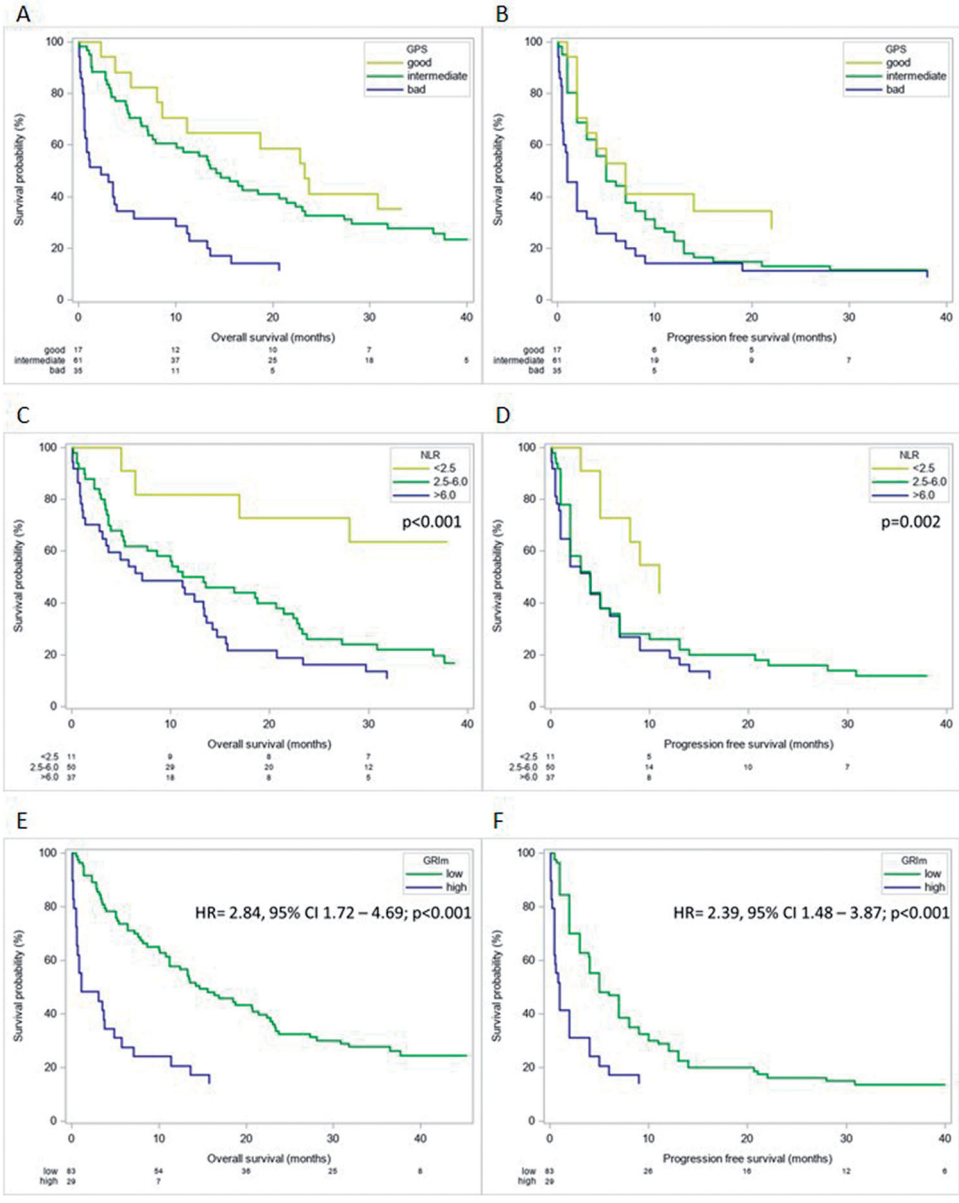


**Figure 4.** Different scores and their influence on outcome parameters in cancer patients. The GPS has prognostic value regarding OS (A) and PFS (B) in our cohort of patients undergoing ICI therapy. Neutrophil to lymphcyte Ratio is strongly prognostic regarding OS (C) and PFS (D) in our cohort of patients. GRIm Score has prognostic value regarding OS (E) and PFS (F) in our cohort of patients treated with ICIs.

group 3: 10.8 months; mPFS group 1: 11 months, group 2: 3.5 months, group 3: 4 months). An increase in NLR was associated with significantly shorter OS and PFS (20% increase: mOS: HR = 1.18, 95% CI 1.09–1.28; $p < .001$; PFS: HR = 1.12, 95% CI 1.04–1.20; $p = .002$; Figure 4(C,D)).

The GRIm Score was calculated for 112 patients; 83 (74%) and 29 (26%) patients had a "low" or "high" score, respectively. Patients with a low score had a significantly better median OS and PFS (14.7 months vs. 1.1 months; and 5 months vs. 1 month, respectively). Furthermore, a high GRIm Score was associated with a shorter OS and PFS (OS: HR = 2.84, 95% CI 1.72–4.69; $p < .001$ and PFS: HR = 2.39, 95% CI 1.48–3.87; $p < .001$; Figure 4(E,F)).

PEV for each score was calculated to compare the performance of our model based on continuous serum parameters with the above-mentioned scores within the first 6 months after start of ICI therapy. GPS explains 14.6% of the variability in overall survival between patients. The continuous NLR accounts for 11.3% and the GRIm Score explains 14.8% of variability in OS, while our multivariable model including LDH, CRP (both with time-dependent effect), Alb and LC

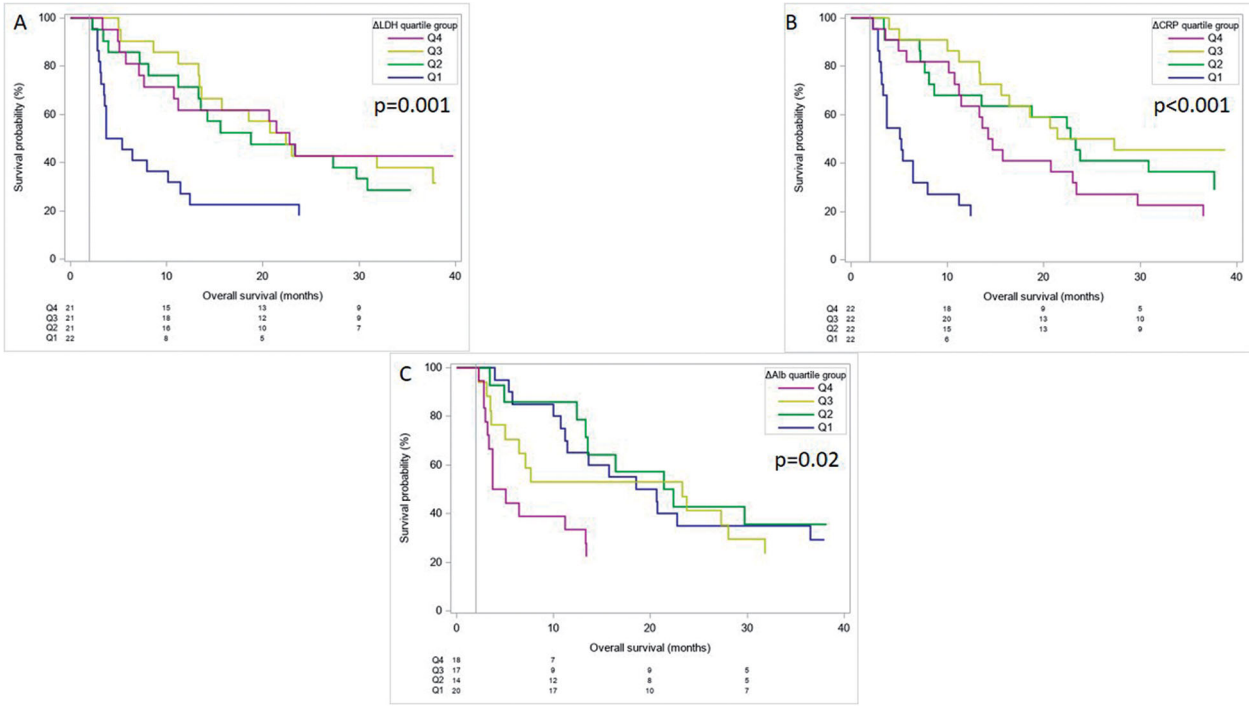


**Figure 5.** OS according to the individual 8-weeks change in LDH (A), CRP (B) and Alb (C) when grouped into quartiles 1–4 (Q1–Q4). Q1 representing the highest increase and Q4 the lowest increase of each serum parameter. $p<.05$ shows a statistically significant effect between all quartiles including a time dependent effect.

explains 29.5% of the variability of the differences in OS between our patients.

### 3.3. Serum parameter changes and association with outcome

Finally, we analysed the effect of a change of the respective parameters within the first 6 – 8 weeks after therapy start on OS and PFS starting from week 8. Patients were grouped into quartiles 1-4 (Q1-Q4) according the individual 8-weeks change in LDH, CRP, and Alb respectively, with quartile group 1 (Q1) representing the highest increase of each serum parameter and subsequently a lower increase or decrease in the other groups.

An increase in LDH remained a factor significantly associated with poor outcome. Q1, which represents patients with the strongest LDH increase during treatment, had a shorter mOS compared to the remaining quartile groups (mOS: Q1 2.6 months, Q2 16.8 months, Q3: 20.4 months, Q4: 20.8 months; $p=.001$; Figure 5(A); Table 4). Patients in Q1 had the worst outcome when grouped according to CRP (mOS: Q1 3.2 months, Q2: 21 months, Q3: 22.4 months, Q4: 12.4 months; $p<.001$; Figure 5(B); Table 4). An increase of Alb was associated with a better outcome (mOS: Q1 17.6 months, Q2 19.9 months, Q3 21.3 months, G4 2.4 months; $p= .02$).

**Table 4.** Median OS and 25–75 percentile and HR and 95% CI for differences between baseline and 8 weeks regarding 6 month – OS for LDH, CRP and Alb.

| LDH | mOS | 25%–75% | LDH | HR | 95% CI |
|---|---|---|---|---|---|
| Q1 | 2.6 | 1.2–10.4 | | | |
| Q2 | 16.8 | 9.2–34.5 | Q2 vs. Q1 | 0.41 | 0.18–0.96 |
| Q3 | 20.4 | 11.4–n.r. | Q3 vs. Q1 | 0.32 | 0.13–0.81 |
| Q4 | 20.8 | 5.7–n.r. | Q4 vs. Q1 | 0.41 | 0.18–0.95 |
| CRP | mOS | 25%–75% | CRP | HR | 95% CI |
| Q1 | 3.2 | 1.2–9.2 | | | |
| Q2 | 21 | 6.1–n.r. | Q2 vs. Q1 | 0.30 | 0.13–0.70 |
| Q3 | 22.4 | 11.4–n.r. | Q3 vs. Q1 | 0.20 | 0.08–0.52 |
| Q4 | 12.4 | 8.8–27.7 | Q4 vs. Q1 | 0.47 | 0.22–0.99 |
| Alb | mOS | 25%–75% | Alb | HR | 95% CI |
| Q1 | 17.6 | 9–n.r. | Q1 vs. Q4 | 0.77 | 0.27–2.17 |
| Q2 | 19.9 | 11–29–n.r | Q2 vs. Q4 | 0.71 | 0.22–2.28 |
| Q3 | 21.3 | 3–29.8 | Q3 vs. Q4 | 1.15 | 0.40–3.33 |
| Q4 | 2.4 | 1.2–11.4 | | | |

Patients were grouped in quartiles according to LDH, CRP or Alb increase from baseline. Q1 represents patients with the highest increase of the respective serum parameter.

Respective KM-curves and HRs are outlined in Figure 5(C) and Table 4.

## 4. Discussion

We showed that both serum parameters (LDH, CRP, Alb or NLR) and prognostic scores (GRIm and GPS) are associated with the outcome of real-world cancer patients receiving ICI regardless of the tumour type.

Implementing those parameters into our model resulted in a higher PEV than one single parameter or score alone.

The advent of ICI led to new therapeutic options in a wide range of tumour types. However, response rates in an unselected population rarely exceeds 20%–30% [14–23]. Recently, the economic impact due to the widespread use of ICIs on health care systems has come more into focus. An excess of costs compared to conventional chemotherapy has been reported by various groups [24,25]. Additionally, patients with an ECOG PS $>$ 1 are rarely included into phase III trials [5,26]. A higher ECOG is associated with a poorer prognosis for cancer patients treated with ICI [5,27]. ECOG PS is highly influenced by the physicians perception and vulnerable to bias [28–31]. As a consequence, more reliable and less observer influenced parameters are needed to assess the prognosis of cancer patients undergoing ICI therapy in the real-world setting.

Increased CRP and LDH levels were associated with a poor outcome in multiple malignancies [32–34], which was confirmed in our study [35–40].

LDH, CRP, Alb and LC were correlated with OS in the in the univariable analysis. A more pronounced increase in LDH or CRP and a decrease in Alb resulted in a worse outcome, indicating that the systemic inflammatory response is prognostic for shorter survival. Similar data was shown in melanoma patients [36]. Interestingly, BMI had no effect on the outcome of our patient collective. Several studies in lung and renal cancer patients showed an association between a higher BMI and superior OS and PFS [41–43]. Especially, Cortellini et al. found that obese patients (BMI $\geq$ 30 kg/m$^2$) had a significantly longer PFS in the ICI cohort (HR = 0.61, 95% CI 0.45–0.82) compared to the chemotherapy cohort (HR = 1.27, 95% CI 1.01–1.60). Additionally, OS for obese patients was superior in the ICI arm (HR = 0.70, 95% CI 0.49–0.99) [44]. In this context, it has to be noted that the inclusion criteria (e.g. TPS $\geq$ 50) or the cut-offs for BMI differed markedly between these studies, while we did not apply cut-off values to BMI before using them as independent variable in our models.

Furthermore, we investigated the prognostic value of GPS, GRIm score and the NLR in our patient cohort.

A strong association with a longer OS and PFS for a good GPS (0 points) compared to an intermediate (1 point) or poor (2 points) score was evident. There are just a few studies in the literature using GPS in ICI treated patients. In two retrospective studies, especially NSCLC patients showed that a good GPS was correlated with an improved PFS [9,45].

The GRIm Score was developed to provide a prognostic tool for patients in Phase I studies and was further tested in NSCLC patients receiving immunotherapy [6–8]. We showed that this tool may be applicable in other solid tumours, since a high GRIm Score was significantly associated with both shorter OS and shorter PFS.

The NLR has been shown to be prognostic in a variety of malignancies and was also used in ICI analysis. A meta-analysis including 12 studies with a total of 1699 patients confirmed that a high pre-treatment NLR is associated with a worse OS in NSCLC patients [46]. Different cut-off values for NLR were used in these studies, ranging from 2.8 to 6.46. We chose a cut-off of $>$6 for the graphical display, because it is used as a component of the GRIm Score and confirmed the results obtained in NSCLC [8]. However, when implemented into our model the continuous NLR change was incorporated. We observed that an increase in the continuous NLR translated into worse OS and PFS (Figure 4).

Finally, it has to be emphasized that our model, which uses the continuous baseline parameters in an optimized linear combination had the highest PEV as outlined above. This indicates a higher prognostic relevance for 6-month OS. The PEV allows to quantify different factors regarding their individual importance to predict outcome, even if different variable types (categorical scores vs. continuous laboratory parameters) or groups of several variables (combination of laboratory parameters) are compared [13]. We acknowledge that the calculated PEV of 29.5% might be overestimated, since it was obtained in the same sample that was used for model estimation, and should be evaluated employing an external validation cohort.

Limitations of this analysis include the retrospective nature and the low sample size. The latter does not allow to generalize negative (i.e. statistically non-significant) results beyond the sample at hand. Although one might assume that the heterogeneous group of different tumour types limits the applicability of this analysis, we have to state again that we included the tumour biology into our statistical model and took this issue into account as described above. Therefore, we demonstrated that pre-treatment serum parameters, the GRIm Score, GPS and NLR have a prognostic role in cancer patients receiving ICI regardless of their underlying malignancy.

Although this analysis was designed as a hypothesis generating exploratory study, which precludes an immediate impact on clinical decision making, this study adds to the evidence of a significant prognostic

role of serum parameters in patients treated with ICI. Based on both the published literature and the results of this study serum parameters should be evaluated in prospective clinical trials as potential prognostic biomarkers in patients treated with ICI.

## Consent

Not applicable. No individual person's personal details, images or videos are being used in this study.

## Ethical approval

This work was approved by the ethics committee of the Medical University of Vienna (2132/2016).

## Author contributions

Minichsdorfer C.: Methodology, Resources, Data curation, Writing original draft, and Final approval

Gleiss A.: Formal Analysis, Validation, Writing, Reviewing and Editing, and Final approval

Aretin M-B.: Resources, Writing, Reviewing and Editing, and Final approval

Wagner C.: Resources, Writing, Reviewing and Editing, and Final approval

Schmidinger M.: Resources, Writing, Reviewing and Editing and Final approval

Fuereder T.: Conceptualisation, Supervision, Project administration, Writing, Reviewing and Editing, and Final approval

All authors agree to be accountable for all aspects of the work

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All other authors stated no conflicts of interest.

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## Data availability statement

Data and materials supporting the results are available upon reasonable request from the corresponding author TF (thorsten.fuereder@meduniwien.ac.at)

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