# Evaluation of Serum Biomarker CEA and Ca-125 as Immunotherapy Response Predictors in Metastatic Non-small Cell Lung Cancer

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**Abstract.** *Background/Aim: Treatment options for advanced non-small cell lung cancer (NSCLC) include immunotherapy. Elevated carcinoembryonic antigen (CEA) and cancer antigen 125 (Ca-125) levels are associated with poorer prognoses of resected NSCLC, but currently no predictive biomarkers exist for immunotherapy response. This study evaluated CEA and Ca-125 as predictive biomarkers for immunotherapy efficiency in patients with metastatic NSCLC. Patients and Methods: The single-centre observational retrospective study includes NSCLC stage III/IV patients treated with programmed death-ligand 1 (PD-L1) inhibitors nivolumab or pembrolizumab. The primary study endpoint was treatment response assessed by CT-scan following RECIST-criteria 1.1. CEA/Ca-125 serum values were determined at initiation of treatment and repeated every 2 weeks. Values closest to the day of CT-scan were compared to baseline values. Results: A total of 136 patients were treated with mono-immunotherapy. Of these, 73 patients were included in the CEA group and 53 patients were included in the Ca-125 group. Baseline CEA and Ca-125 ranged from 8.14 to 5,909 and 1.1 to 4,238 respectively. The sensitivity for Ca-125 as predictor for tumor response was 62.9% (95% CI=61.8%-63.6%), specificity 61.1% (95% CI=60.2%-62.0%), with a positive predictive value (PPV) of 75.9% (95% CI=75.2%-76.7%). For CEA, the sensitivity was 72.0% (95% CI=71.5%-72.5%), specificity 47.1% (95% CI 46.4%-47.8%), with a PPV of 80.0% (95% CI=79.6%-80.4%). Conclusion: Increased serum CEA might predict tumor progression in NSCLC patients treated with PD-L1 inhibitors.*

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*Key Words:* NSCLC, Immunotherapy, PD-L1, Ca-125, CEA.

*Unconfirmed progression accompanied by increased CEA would support discontinuation of the immunotherapy, while continuation would be advised when serum CEA is not increased.*

Cancer is still the second cause of death worldwide, and lung cancer is the leading cause of cancer-related deaths (1). The incidence of lung cancer is still increasing (2). A total of 85% of all cases of lung cancer are non-small cell lung cancer (NSCLC). However, 30-40% of NSCLC present with metastatic disease at the time of diagnosis (stage IV) (3, 4). The overall 5-year survival for NSCLC is very poor less than 20%, and even worse in stage IV (5). Only 20% of all lung malignancies qualify for surgical resection. The cornerstone treatment for advanced/metastatic disease was platinum doublets therapy. Recently this has changed because of the discovery of immunotherapy as a treatment option for stage III and IV NSCLC (6).

The most recent ESMO guideline indicates immunotherapy as a first-line treatment option for all non-oncogene driven NSCLC, both adeno- and squamous cell carcinoma (7-11). Programmed death-ligand 1 (PD-L1) tumor expression indicates whether immunotherapy alone or combined with chemotherapy should be initiated (12). Although PD-L1 has been shown to be associated with immunotherapy response, the search for better biomarkers that identify responders prior to treatment continues.

For response evaluation of lung cancer treatment, CT-imaging using the RECIST-criteria assessment is the golden standard for solid tumors (13). Recently, special immune-related response criteria in solid tumors (iRECIST) have been established. A newly implemented term in the Immuno-RECIST criteria (compared to the original RECIST criteria) is "unconfirmed progressive disease". Unconfirmed progression means that progression is observed compared to the baseline tumor by imaging. This may reflect inflammatory swelling of

the tumor due to an effective immune response, but can also simply represent progressive growth of the tumor. At the stage of unconfirmed progression, the therapy is continued.

Confirmed progressive disease requires confirmation by subsequent imaging after 4-8 weeks. The therapy will then be discontinued (14). Biomarkers that are able to predict the response to immunotherapy during treatment advance of radiological progression would allow early discrimination between responders and non-responders, which – in turn – could guide early discontinuation of ineffective treatment. An accurate biomarker could help decide to continue or discontinue treatment with PD-L1 inhibitors. Unconfirmed progression accompanied by an increase of an accurate biomarker would support discontinuation of the immunotherapy. Conversely, if the biomarker does not show an increase, continuation of the immunotherapy would be rational. Unfortunately, there are currently no such predictive biomarkers available in clinical practice.

In a recent case report, de Jong *et al*. suggested the possible clinical utility of carcinoembryonic antigen (CEA) serum levels for evaluation of immunotherapy response in NSCLC (15). CEA is a glycoprotein produced in the gastrointestinal tract, the pancreas and liver. CEA is clinically used as a tumor marker in colon carcinoma (16). Cancer antigen 125 (Ca-125) is a glycoprotein mostly produced in fetal tissue. Abnormal Ca-125 levels (found in fluids of different origins such as ascites, pleura, pericardium, amniotic fluid, cyst fluid, bronchoalveolar fluid) can derive from irritation. Peritoneal Ca-125 significantly contributes to circulating Ca-125 concentrations, leading to elevated Ca-125 values. Differences in serum CA 125 found in malignant or benign diseases may be related to the number of cells that produce the marker. Ca-125 is commonly used as a tumor marker in ovarian cancer (17, 18).

CEA- and Ca-125 are also implicated as biomarkers in NSCLC. Several studies identified elevated levels of CEA (19-32) or Ca-125 (27-32) at baseline to be associated with poor prognosis in resected NSCLC. However, none of these studies involved immunotherapy in the context of advanced NSCLC. Therefore, it appears of interest to examine the value of Ca-125 and CEA levels at the start of -and during- nivolumab and pembrolizumab therapy to predict response to immune checkpoint therapy in late stage NSCLC and to select responders and non-responders. The aim of the study was to evaluate CEA and/or Ca-125 as early predictive biomarkers (markers) for treatment response in patients with NSCLC treated with immunotherapy.

## Patients and Methods

*Subjects*. In this single centre observational cohort study, all patients diagnosed with NSCLC stage IIIB or IV treated with PD-L1 inhibitors (nivolumab or pembrolizumab) at the St. Antonius hospital, Nieuwegein, the Netherlands between September 2015 and August 2018. Nivolumab was used at a dose of 240 mg every 2

weeks, from August 1st 2018, administered every 4 weeks and pembrolizumab at a dose 200 mg every 3 weeks.

*Data collection and study design*. Individual patient data (clinical, pathological, treatment history, serum value CEA/Ca-125) were manually collected from electronic medical records. Baseline was defined as the date of the first dose of immunotherapy. The study was approved by Medical Research Ethics Committees United (MEC-U) (Registration No: W18.226). Treatment response, defined as progressive disease or stable disease/partial response (combined). Response was assessed using the RECIST-criteria 1.1 based on radiological examination by means of CT-scans. This was performed at 6 weeks after (every) 3 courses of nivolumab, after 1 August 2018 every 8 weeks, and at 6 weeks after 2 courses of pembrolizumab. All patients without radiological examination were excluded.

The serum value of CEA and Ca-125 was measured at the start of immune therapy and with each consecutive cycle, by a blood chemistry analyser (the COBAS 3000). Patients without a CEA and/ or Ca-125 serum value within 2 weeks of start date with immunotherapy were excluded. Patients without a CEA and/or Ca-125 serum value during the period of 2 weeks before or 2 weeks after the first scan that reveals progressive disease (PD) or partial response (PR) were excluded.

If the CT-scan did not reveal PD or PR during follow-up, we used the CEA and Ca-125 serum values obtained closest to the latest CT-scan revealing SD. Furthermore, patients without a CEA and/or Ca-125 serum value within 2 weeks of latest CT-scan revealing SD were excluded.

Other retrospectively collected patient characteristics from medical records are the following: smoking status, histology (adeno-squamous carcinoma) line of therapy (1st or 2nd), Eastern Cooperative Oncology Group (ECOG) performance status, PD-L1 expression (<1%/<49%/>50%), and immune-related adverse events. If not noted in medical history, this was registered as unknown.

*Statistical analysis*. Standard descriptive statistics were used to report the data on patient characteristics. Diagnostic test characteristics of CEA and Ca-125 for tumor response were calculated from 2×2 tables. Different cut-off points for increase or decrease of the biomarkers CEA and Ca-125 were evaluated. Increase or decrease in general and specifically increase or decrease of 10%, 15% and 20% were evaluated. Positive predictive value, negative predictive value, sensitivity and specificity were calculated using standard methods (33).

To determine the performance of CEA and Ca-125 as a biomarker for response we plotted a ROC (receiver operator characteristic) curve. Using the percentage change as a continuous value (not as a binomial indicator). In addition, the correlation between CEA and Ca-125 was analysed by plotting the delta log (between start of immunotherapy and the moment that the CT-scanning revealed PD or non PD) of CEA and Ca-125.

## Results

*Patient population*. A total of 136 patients diagnosed with NSCLC stage IIIB or IV who were treated with PD-L1 inhibitors (nivolumab or pembrolizumab), between December 2015 and August 2018 according to pharmacy reports. Eighteen patients were excluded because the patient died before CT-scan (n=11), inadequate performance stats to
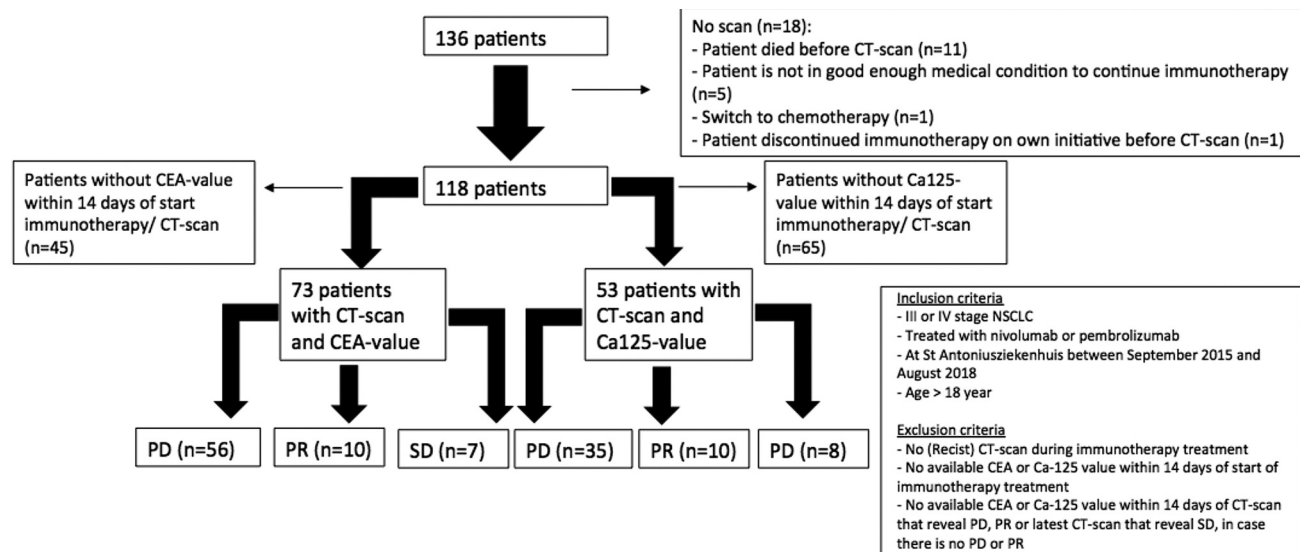
Figure 1. *Study flowchart. Ca-125: Cancer antigen 125, CEA: carcinoembryonic antigen, PD: progressive disease, PR: partial response, SD: stable disease.*

continue treatment before the first CT-scan (n=5), a switch to chemotherapy before first CT-scan (n=1), or because discontinuation of immunotherapy on own initiative before first CT-scan (n=1). For 45 patients, no CEA value was determined either within 2 weeks of immunotherapy start date, or within 2 weeks of the first scan that revealed PD or PR within two weeks of the last CT-scan that reveals SD (if during follow-up the CT-scan did not reveal PD or PR). For 65 patients, no Ca-125 value was determined within 2 weeks of immunotherapy start date, or within 2 weeks of the first scan that revealed PD or PR within two weeks of the last CT-scan that reveals SD (if during follow-up the CT-scan did not reveal PD or PR). The inclusion of patients is depicted in Figure 1.

Finally, 73 patients were included for CEA as biomarker and 53 patients were included for Ca-125 as biomarker for predicting tumor progression. Baseline data of the study population is shown in Table I. Baseline CEA and Ca-125 ranged from 8.14 to 5909 and 1.1 to 4238 respectively.

*Predictive value of CEA and/or Ca-125 as predictor for disease progression.* Different cut-off points for increase or decrease of biomarkers CEA and Ca-125 (10%, 15% and 20%) were evaluated. None of the cut-off points improved the diagnostic value compared to simple increase or decrease. The results are shown in the appendix. Figure 2 reveals the predictive value of any increase or decrease (cut-off 0%) from baseline when compared to the first-time PD, PR or the last CT-scan with SD (when the CT-scan did not reveal PD or PR during follow-up).

For Ca-125 increase or decrease as a predictive test for tumor response [based on the CT-scan using the (i) RECIST-

criteria 1.1] leads to the following results: sensitivity was 62.9% (95% CI=61.8%-63.6%), specificity 61.1% (95% CI=60.2%-62.0%), a positive predictive value (PPV) of 75.9% (95% CI=75.2%-76.7%), a negative predictive value (NPV) of 45.8% (95% CI=44.9%-46.7%).

For CEA as predictive test for tumor response [based on the CT-scan using the (i)RECIST-criteria 1.1] leads to the following results: sensitivity was 72.0% (95% CI=71.5%-72.5%), specificity 47.1% (95% CI=46.4%-47.8%), a PPV of 80.0% (95% CI=79.6%-80.4%), an NPV of 28.6% (95% CI=28.1%-29.1%).

*ROC of CEA and Ca-125.* For CEA, 73 patients were analysed (56 with PD and 17 patients with non-PD). The AUC (area under the curve) was 0.6487 (CI=0.526-0.771). For Ca-125, 53 patients were analysed (35 with PD and 18 patients with non-PD). The AUC: 0.5871 (CI=0.424-0.751). The analyses are shown in Figure 3, represented by receiver operator characteristic (ROC) curves. To that end, we applied the percentage change as a continuous value (and not as a binomial indicator). These numbers agreed with the previous conclusions that these biomarkers (CEA and Ca-125) had a relatively high positive predictive value and a much poorer negative predictive value.

*Correlation between the levels of CEA and Ca-125.* Correlation of CEA and Ca-125 levels have been reported for ovarian cancer (34). We therefore determined a possible correlation of the levels of CEA with those of Ca-125 between start of immunotherapy and PD or non-PD. To this end, we plotted the delta log (between start of immunotherapy and the moment that

Table I. *Table of baseline characteristics.*

|  | No. of patients total, 136 | No. of patients included CEA, 73 | No. of patients included Ca-125, 53 |
|---|---|---|---|
| Age, median (range, year) | 66 (44-82) | 67 (45-79) | 67 (51-77) |
| Gender Male (%) | 68 (50.0) | 32 (43.8) | 25 (47.2) |
| Smoke status (%) |  |  |  |
| Current | 21 (15.4) | 9 (12.3) | 9 (17.0) |
| Former | 66 (48.5) | 38 (52.1) | 26 (49.1) |
| Non | 4 (2.9) | 2 (2.7) | 1 (1.9) |
| Unknown | 45 (33.1) | 24 (32.9) | 17 (32.1) |
| ECOG performance status (%) |  |  |  |
| 0 | 29 (21.3) | 18 (24.7) | 14 (26.4) |
| 1 | 95 (69.9) | 46 (63.0) | 36 (67.9) |
| 2-3 | 3 (2.2) | 0 (0.0) | 0 (0.0) |
| Unknown | 9 (6.6) | 7 (9.6) | 3 (5.7) |
| Histology (%) |  |  |  |
| Adenocarcinoma | 94 (69.1) | 53 (72.6) | 35 (66.0) |
| Squamous | 28 (20.6) | 13 (17.8) | 13 (24.5) |
| Other diagnose | 3 (2.2) | 1 (1.4) | 1 (1.9) |
| Unknown | 11 (8.1) | 6 (8.2) | 4 (7.5) |
| Stage (%) |  |  |  |
| IIIB | 78 (57.0) | 40 (54.8) | 26 (49.1) |
| IV | 58 (43.0) | 33 (45.2) | 27 (50.9) |
| EGFR mutation (%) | 57 (56.4) | 33 (56.9) | 19 (52.8) |
| Not determined (%) | 35 (25.7) | 25 (43.1) | 17 (47.2) |
| Prior line of therapy (%) |  |  |  |
| 1st line | 15 (11.0) | 7 (9.6) | 7 (13.2) |
| 2nd line | 119 (87.5) | 65 (89.0) | 45 (84.9) |
| Unknown | 2 (1.5) | 1 (1.4) | 1 (1.9) |
| PD-L1 expression (%) |  |  |  |
| <1% | 16 (11.8) | 6 (8.2) | 4 (7.5) |
| <49% | 8 (5.9) | 5 (6.8) | 2 (3.8) |
| >50% | 32 (23.5) | 15 (20.5) | 11 (20.8) |
| Not available | 80 (58.8) | 47 (64.4) | 36 (67.9) |
| Immunotherapy (%) |  |  |  |
| Nivolumab | 107 (78.7) | 59 (80.8) | 43 (81.1) |
| Pembrolizumab | 29 (21.3) | 14 (19.2) | 10 (18.9) |
| Number of cycles, median, (IQ) | 4 (2-8) | 6 (3-13) | 7 (4-18) |
| Immune related adverse events (%) | 17 (12.5) | 8 (11.0) | 8 (15.1) |
| Gastro-intestinal | 11 (8.1) | 2 (2.7) | 3 (5.7) |
| Hypothyroidism | 4 (2.9) | 3 (4.1) | 3 (5.7) |
| Pneumonitis | 3 (2.2) | 2 (2.7) | 2 (3.8) |
| Cerebral | 1 (0.7) | 0 (0.0) | 0 (0.0) |
| Cutaneous | 1 (0.7) | 1 (1.4) | 0 (0.0) |
| Nephritis | 1 (0.7) | 0 (0.0) | 0 (0.0) |

ECOG: Eastern Cooperative Oncology Group, EGFR: epidermal growth factor receptor, PD-L1: programmed death-ligand 1.

the CT-scanning revealed PD or non-PD) of CEA on the X-axis and Ca-125 on the Y-axis (Figure 4). This revealed a significant number of outliers, resulting in a poor correlation between the levels of CEA and Ca-125 in individual patients.

## Discussion

We observed a relatively high positive predictive value of 80% for CEA. We conclude that an increase of CEA has a relatively

high positive predictive value for PD. Vice versa, the meaning of a decrease in CEA level during immunotherapy does not hold a high predictive value. These results confirm the association between an increase of serum CEA and PD. This suggests that an increase of serum CEA might be a reliable biomarker as a predictor for immunotherapy in NSCLC patients. The results for Ca-125 are comparable (but less pronounced).

Since 2015, immune checkpoint inhibitors such as anti-PD1 represent a widely used treatment option for end-stage
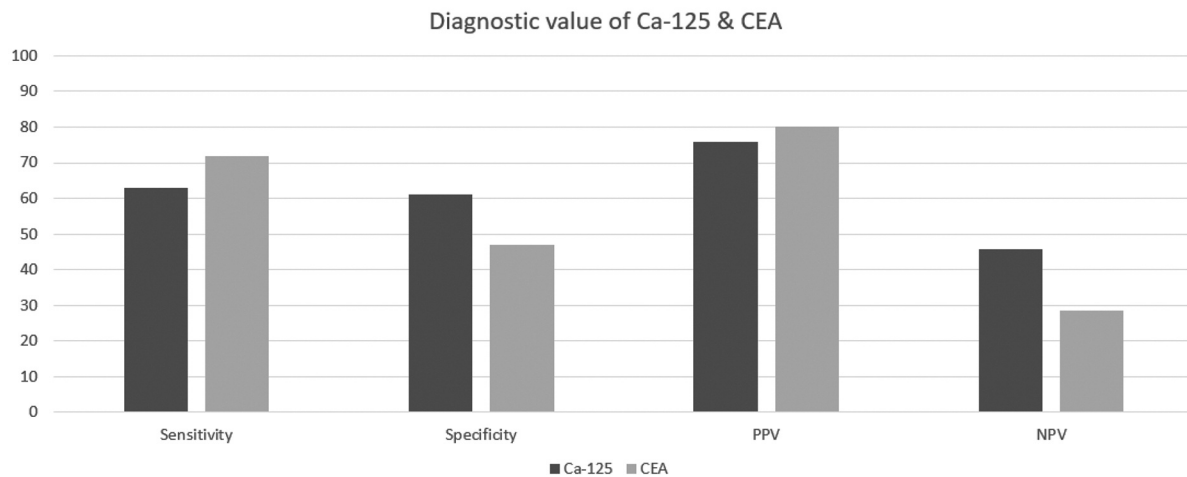
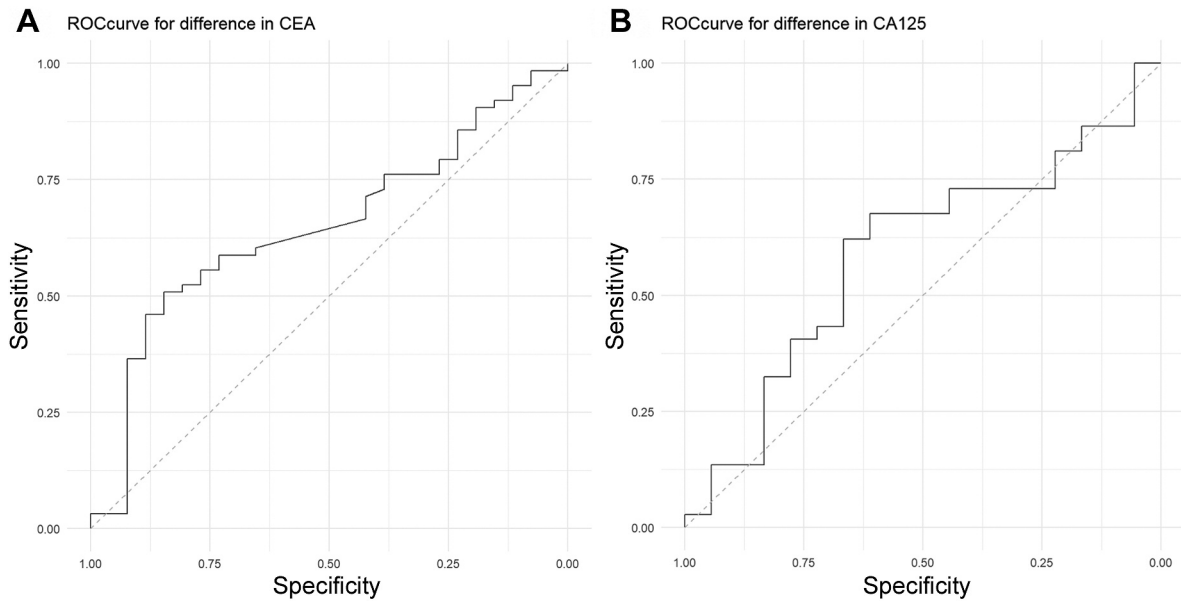Figure 2. *Diagnostic value of cancer antigen 125 (Ca-125) and carcinoembryonic antigen (CEA).*



Figure 3. *ROC curve for carcinoembryonic antigen (CEA) (A) and cancer antigen 125 (Ca-125) (B).*

cancers such as NSCLC stage IIIB or IV (7-9). However, many patients do not respond to anti-PD1 treatment while the immune-related adverse event can give severe toxicities. In a subset of patients, this treatment leads to survival benefits. Biomarkers that are able to predict the response at the start of, or during immunotherapy would allow early discrimination between responders and non-responders, which would prevent inefficient and extremely expensive therapy. Such biomarkers would be able to guide the decision-making process to switch to a different treatment.

Unfortunately, there are currently no predictive biomarkers available that have been validated in clinical practice.

CEA is a glycoprotein produced in the gastrointestinal tract, the pancreas and liver. CEA is clinically used as a tumor marker in colon carcinoma (26). CEA has also been used as a monitoring tool for therapy in advanced-stage lung carcinoma. Most studies have reported elevated levels of CEA to be associated with poor prognosis in resected NSCLC including stage I (19-25). A smaller number of studies have reported no such association (26-31). In
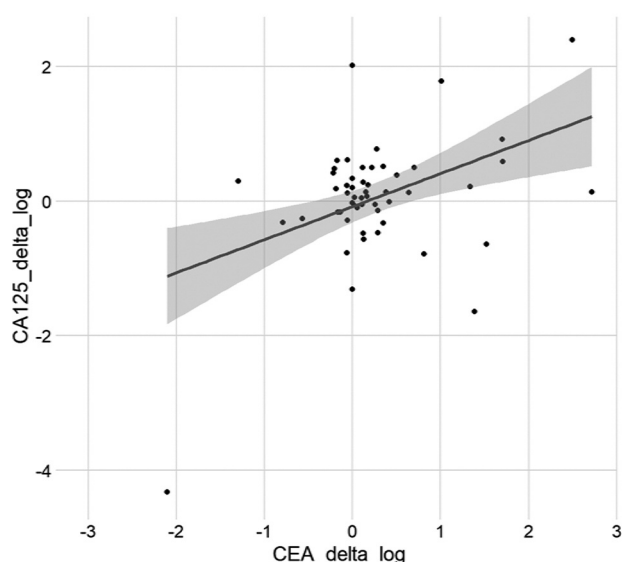
Figure 4. *Correlation between differences in carcinoembryonic antigen (CEA) versus cancer antigen 125 (Ca-125) in PD- and non-PD-patients. Delta log of CEA on the X-axis and Ca-125 are plotted on the Y-axis. The linear regression line is drawn, in grey the CI of 95% is indicated.*

addition, some reports showed changes in CEA level during chemotherapy (35, 36) and targeted therapy (37-38) and reported a higher predictive value compared to baseline value alone during treatment. Thus, there still appears that no consensus exists about the value of CEA as a prognostic marker in NSCLC (26).

Ca-125 is -like CEA- a glycoprotein that is mostly produced in fetal tissue, but in addition is produced in mesothelial tissues in adults. Ca-125 is commonly used as a tumor marker in ovarian cancer. The following 3 studies report a worse prognosis of NSCLC when Ca-125 is positive as a pre-operative marker (27-32). Of relevance here, none of these studies involved immunotherapy. It appears very interesting to evaluate the value of Ca-125 and CEA levels at the start of -and during- nivolumab and pembrolizumab therapy to predict response to immune checkpoint therapy in late stage NSCLC and to select responders and non-responders.

To the best of our knowledge, this is the first study focusing on the role of CEA and Ca-125 as a potential biomarker for tumor response in advanced NSCLC treated with immunotherapy. In agreement with the studies investigating the predictive value of the increase of CEA for chemotherapy (35-36) and targeted therapy (37, 38), we observed a relatively high positive predictive value of 80%.

Our results are the first indication that there is a predictive role for increase in CEA level and to lesser extent in Ca-125 level for predicting tumor progression in patients diagnosed with advanced-stage NSCLC treated with immunotherapy.

Our study also describes a poor correlation of the differences in CEA and Ca-125 levels between start of immunotherapy and the moment that the CT-scanning revealed PD or non-PD. For this, it appears that combining measurements of CEA with Ca-125 carries little added value over measuring CEA alone.

This study has several strengths. First, the single-centre approach has ensured that all clinical decisions and measurements, as well as the biomarker serum analysis were performed consistently during the entire follow-up period for all the patients. Second, we consistently used the gold standard for evaluating immunotherapy response by CT-scan (i)RECIST-criteria 1.1 and compared this with the two potential biomarkers. Third, we are the first to evaluate the correlation between CEA and Ca-125 in immunotherapy-treated patients with NSCLC.

Some limitations of this study need to be discussed as well. First, the design of this study is a single-centre retrospective study. This may lead to a patient selection bias so it could be possible that this study does not completely represent the average advanced stage NSCLC patient. Second, we had to exclude around 50% of the study population because biomarker values were not measured at time points predefined in our study. Yet, we note no obvious differences in baseline measures (Table I) between the groups of all patients and included patients.

If validated in future studies, CEA may be useful to physicians to make a clinical decision based on the early recognition of responders and non-responders. If an increased CEA is apparent, immunotherapy can be interrupted early without the necessity of a 2nd CT-Scan, confirming ''the unconfirmed progression''. This would be beneficial because of the poor survival outcome and low probability of controlled disease. The advantages for the patient would be the avoidance of severe toxicities and reduction of radiation load. Additionally, this would avoid the cost of immunotherapy and imaging.

We conclude that an increase of serum CEA might be a reliable biomarker to predict tumor progression in NSCLC patients treated with immunotherapy. Serum CEA might thus advise continuation or discontinuation of treatment with PD-L1 inhibitors: Unconfirmed progression accompanied by an increase of CEA would support discontinuation of the immunotherapy, while continuation of the immunotherapy would be advised when serum CEA is not increased. Further prospective studies in larger populations will need to be performed to confirm a predictive value of an increased CEA level related to PD in advanced stage NSCLC patients treated with immunotherapy.

## Conflicts of Interest

The Authors declare no conflicts of interest.

## Authors' Contributions

MR Clevers: Methodology, validation, formal analysis, investigation, resources, data curation, visualization, writing, project administration. FMNH Schramel: Conceptualization, methodology, supervision. EA Kastelijn: Conceptualization, methodology, supervision. BJM Peters: Conceptualization, methodology, supervision. H Kelder: Methodology, formal analysis, visualization.

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