Feasibility of ApoC1 serum levels as tumor biomarker in glioblastoma patients: a pilot study

Michelle Hilbert1, Peter Kuzman2, Wolf C. Mueller2, Jürgen Meixensberger1 & Ulf Nestler

Apolipoprotein C1 (ApoC1) has been detected immunohistochemically in glioblastoma tissue, probably expressed by activated monocytes and microglia. The present study was conceived to determine whether the amount of intratumoral ApoC1 expression leads to measurable changes of serum levels after glioblastoma resection or during recurrence. 176 blood samples from 70 glioblastoma patients were collected perioperatively and during subsequent therapy. ApoC1 serum levels were determined using an enzyme linked immunosorbent assay (ELISA). High absorption values due to lipemic or hemolytic serum were removed from the final dataset using a stem and leaf plot. Samples were grouped according to the treatment stage to compare mean ApoC1 serum levels. The number of patients with falling or increasing perioperative values was assessed. 167 ApoC1 serum values from 68 glioblastoma patients were amenable to statistical evaluation. Mean ApoC1 serum level was 91.9 µg/ml (n = 167, sd = 36.0). In samples from patients undergoing first glioblastoma resection, the mean preoperative value was significantly higher (94.8 µg/ml, n = 37, sd = 29.5) than after surgery (77.4 µg/ml, n = 41, sd = 23.2, p = 0.009). Individually, falling ApoC1 levels were detected in 25 and rising levels in 9 patients (p = 0.0061). Single absolute serum levels of ApoC1 do not allow an estimation of glioblastoma activity or tumor response. Although pathophysiologically of interest, ApoC1 serum levels did not qualify as a potential biomarker in glioblastoma management. Our results do not seem to encourage larger, multicenter studies.

Abbreviations
ALAT Alanine-aminotransferase
ASAT Aspartate-aminotransferase
ApoC1 Apolipoprotein C1
CRP C-reactive protein
ELISA Enzyme linked immunosorbent assay
IDH Isocitrate dehydrogenase
µg/ml Microgram per milliliter
mRNA Messenger ribonucleic acid
MRI Magnetic resonance imaging
n Number
sd Standard deviation

Mass spectrometric analysis has shown the presence of apolipoprotein C1 (ApoC1) in glioblastoma cyst fluid1. This finding was confirmed in solid glioblastoma tissue by assessment of mRNA expression and by immunohistochemical staining2.

ApoC1 is mainly synthesized by liver cells to be integrated into high density lipoproteins and exerts a blocking function on low density lipoprotein receptors. The protein is coded on chromosome 19q13.32 and is expressed in several splice variants, mainly with a size of 6631 Dalton1. Astrocyte-based expression of ApoC1 in the central nervous system has been described3. The ApoC1 gene is expressed when monocytes differentiate into macrophages.

Suppression of toll-like receptor dependent activation of microglia by ApoC1 has been observed, hypothetically constituting an autocrine loop for modulation of immune response or even enabling an immune escape of...
glioblastoma cells. Consequently, higher ApoC1 expression could correspond to larger glioblastoma volume or recurrent growth of tumor cells. On the other hand, intratumoral necrosis is a histologic feature of malignant glioma and involves large amounts of phagocytic cells. Since up to 40% of glioblastoma tissue consists of activated monocytes or microglia, detectable amounts of ApoC1 in glioblastoma samples can occur. From this point of view, ApoC1 expression would mirror the activity of tissue macrophages.

Theoretically, due to the tumor-induced damage of the blood brain barrier, and after neurosurgically provoked local disruption, ApoC1 may be swept into the blood stream and consecutively lead to measurable changes of ApoC1 serum levels. Accordingly, the first assessment in neurosurgical patients had disclosed a significant drop of ApoC1 serum levels after the surgical intervention. Here we present a detailed analysis of serum samples exclusively from glioblastoma patients, examined using a commercially available enzyme linked immunosorbent assay (ELISA). A correlation of these values to tumor volume or the clinical course of the patients would then eventually allow the use of ApoC1 as serum biomarker for glioblastoma.

Patients and methods

The study protocol, procedures and patient informed consent form had been approved by the local ethics committee prior to the study (311/19-ek, Medizinische Fakultät Ethik-Kommission Leipzig). 176 serum samples from 70 glioblastoma patients were collected prospectively before and after neurosurgical interventions, as well as from patients presenting to the outpatient clinic during follow-up visits for chemotherapy.

Follow-up visits were routinely scheduled in 3-months intervals with the neurosurgical intervention as starting point. The routine serum samples for leukocyte and thrombocyte counts during chemotherapy were taken both during concomitant chemotherapy or during maintenance cycles. According to the patients course, the sampling time points varied from 1 to 227 months postoperatively (mean 18.7 months, median 10 months).

Prospectively collected samples were frozen at –20 °C until assessment by enzyme linked immunosorbent assay (EHAPOC1, Invitrogen, Waltham, MA) according to the instructions of the manufacturer. Samples were assessed in duplicate and in two dilutions (1:10.000 and 1:50.000). The 96-well plate was read twice in a photometric microplate reader and control absorption at 550 nm was subtracted from the 450 nm absorption values. The 8 obtained values were averaged and expressed in µg/ml.

Demographic data of the patients were extracted from the digitally archived records. To assess a potential influence of liver function or perioperative blood loss on ApoC1 concentration, serum values for ALAT, ASAT, bilirubin, C-reactive protein, hemoglobin and leukocyte count were obtained from the sample or the same day in the serum sample. In order to examine the ApoC1 correlation to tumor burden, MRI tumor volume [ml] on imaging corresponding to the date of serum withdrawal was calculated. The formula of a rotational ellipsoid ($0.52 \times A \times B \times C$) with assessment of the three diameters A, B and C [cm] in axial, sagittal and coronal T1 weighted MRI after application of contrast medium was employed.

Evaluation of data was performed using SPSS 27 (IBM, Armonk, NY). Outliers and extreme values due to lipemic or hemorrhagic contamination of ApoC1 samples were identified by stem-and-leaf plot and removed. Statistical significant differences for mean values were assessed by Mann–Whitney-U test and for categorical variables by chi-square test. Parameters with significant differences between preoperative and postoperative mean values were correlated to the ApoC1 serum levels based on individual samples, and diagnosis of proportionality was assessed by linear regression.

Ethical approval. The collection of specimens, data acquisition and experimental procedures had been approved by the local ethics committee (311/19-ek, Ethik-Kommission der Medizinischen Fakultät der Universität Leipzig) and were performed in accordance with the ethical standards of the Declaration of Helsinki and its later amendments. All patients have given informed consent prior to inclusion into the study.

Results

After removal of 9 lipemic/hemorrhagic samples with ApoC1 extreme values over 227 µg/ml according to the SPSS-based stem-and-leaf plot, the remaining dataset comprised 167 samples from 68 patients with a mean age of 64.06 years and an even distribution between men and women (34 each). 37 samples were obtained before the first neurosurgical intervention for glioblastoma, 8 before a second, and 3 before a third surgery. 67 specimens were sampled during chemotherapy. Histologically, in 64 patients the tumor was diagnosed as glioblastoma IDH-wild type, in one as gliosarcoma IDH-wild type, and in three glioblastomas an IDH mutation was disclosed. Histologic assessment refers to the 2016 revision of the 4th edition of the WHO classification of central nervous system tumors, since sampling of tumor specimens ended in December 2020.

There was no statistical difference in mean ApoC1 serum levels, neither concerning patient characteristics as gender, age or months after neurosurgical resection, nor in view of ELISA characteristics such as timing of the test after the start of the study or seasonal influences.

Concerning the first neurosurgical intervention for glioblastoma, the mean preoperative ApoC1 serum level was significantly higher than the mean postoperative value (Table 1). Similarly, the mean postoperative ApoC1 serum level was significantly lower than the level during chemotherapy. These findings were confirmed when additionally including patient samples from second or third interventions. Also, on an individual patient base, significantly more patients had lower ApoC1 serum values after surgery than before.

When assessing the potential confounding factors, mean postoperative values were found to be significantly lower for MRI tumor volume and hemoglobin. In contrast, serum levels for bilirubin and CRP were rising (Table 2). There were no statistically significant perioperative differences concerning ALAT, ASAT or leukocyte count. Of note, when assessing the individual patient serum pairs by linear regression, no proportionality between ApoC1 changes and the four other parameters was detected (Table 2).
During the follow-up period, 24 patients succumbed to their disease. In the 58 according samples, the mean ApoC1 level had been 93.79 µg/ml (SD 37.69), not differing from the mean level in the study group. The relationship of ApoC1 levels to survival time was then assessed by linear regression analysis. Neither relating single absolute ApoC1 values to remaining life span, nor relating averaged values per patient to survival time from first surgery, showed a significant correlation (Fig. 1).

In 33 patients receiving chemotherapy, multiple sequential ApoC1 levels were available. Only in 5 (15.2%) of these patients the last obtained sample during follow-up showed the highest ApoC1 level, no correlation to treatment stage or clinical course was disclosed.

In another subset of 33 patients, tissue slices were assessed immunohistochemically for ApoC1 expression, using a semiquantitative scale. Only one slide was scored as negative, the corresponding serum sample showed an ApoC1 value of 87.06 µg/ml. The highest staining score was also found only once, with a corresponding serum value of 64.84 µg/ml. Low staining scores were attributed in 8 cases, medium scores in 18 and high scores in 5 slides. A significant correlation of the immunohistochemical ApoC1 expression to serum levels or to survival time was not found.

**Discussion**

In the present cohort, a significant decrease of mean ApoC1 serum levels was detected after glioblastoma resection. In accordance with this, significantly more patients had falling ApoC1 serum values after neurosurgery than rising values.

A correlation to confounding medical factors, such as reduction of tumor volume, anesthesia induced changes in liver function, dilution effects or post-aggression metabolism was excluded.

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**Table 1.** Mean ApoC1 levels and changes in individual patients after surgery for glioblastoma. Values with the same superscript letter differ significantly (*p* < 0.05 Mann–Whitney-U test). SD = standard deviation. First neurosurgery = only patients undergoing their first intervention for glioblastoma. All = including patients with first, second or third intervention for glioblastoma.

| Samples                          | Number | Mean [µg/ml] | SD   |
|----------------------------------|--------|--------------|------|
| Whole group                      | 167    | 91.9         | 36.0 |
| Preoperatively (first neurosurgery) | 37     | 94.8*        | 29.5 |
| Postoperatively (first neurosurgery) | 41     | 77.4**       | 23.2 |
| Preoperatively (all)             | 48     | 95.2*        | 33.7 |
| Postoperatively (all)            | 52     | 78.3**       | 23.4 |
| During chemotherapy              | 67     | 100.1**      | 42.5 |

**Table 2.** Perioperative changes in ApoC1 level, tumor volume, bilirubin, CRP and hemoglobin. *p* < 0.05 Mann–Whitney-U test, *p* < 0.05 chi-square test.

| Parameters with significant differences between preoperative and postoperative values after first neurosurgical intervention | Mean values | MRI tumor volume | Bilirubin | CRP | Hemoglobin |
|--------------------------------------------------------------------------------------------------------------------------|-------------|-----------------|-----------|-----|------------|
| Mean values                                                                                                               | ApoC1 [µg/ml] | [ml]            | [µmol/l]  | [mg/l] | [mmol/l]   |
| Normal range                                                                                                              | See Table 3  | 0               | < 17.1    | < 5   | 7.5–9.9    |
| Preoperative                                                                                                              | 94.8        | 35.5            | 7.2       | 3.2   | 8.7        |
| Postoperative                                                                                                              | 77.4*       | 11.8*           | 10.2*     | 22.2* | 7.6*       |
| Change in %                                                                                                                | − 18.4      | − 66.8          | 32.5      | 593.8 | − 12.6     |

| Patient number (n = 34)                                                                                                       | Falling | Increasing | Chi-square | p   |
|------------------------------------------------------------------------------------------------------------------------------|---------|------------|-------------|-----|
| Preoperative                                                                                                                | 25      | 34         | 6           | 2   | 33         |
| Postoperative                                                                                                               | 9#      | 0#         | 26#         | 17# | 1#         |
| Change in %                                                                                                                | 15      | 15         | 15          | 15  | 15         |
| Correlation to ApoC1 (linear regression)                                                                                     | No      | No         | No          | No  | No         |

| Potential confounder                                                                                                          | Resection | Anesthesia-induced | Post-aggression metabolism | Dilution effect |
|------------------------------------------------------------------------------------------------------------------------------|------------|-------------------|-----------------------------|-----------------|
| Correlation to ApoC1 (linear regression)                                                                                     | No         | No                | No                          | No              |

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**Table 2.** Perioperative changes in ApoC1 level, tumor volume, bilirubin, CRP and hemoglobin. *p* < 0.05 Mann–Whitney-U test, *p* < 0.05 chi-square test.
However, the lack of correlation of ApoC1 serum values to tumor volume on MRI and to protein expression in histological slides, as well as the missing relationship of ApoC1 levels to survival time do not favor the use of ApoC1 as serum biomarker or prognostic marker for glioblastoma.

Our results in glioblastoma patients mirror the ongoing discussion in current publications concerning malignant diseases (Table 3). Two studies dealing with gastric cancer found higher ApoC1 serum levels to be associated with a better prognosis. In contrast, another recent publication described the tumor burden in gastric cancer to be elevated in the presence of higher ApoC1 levels. An analysis in pancreatic adenocarcinoma stated that ApoC1 serum levels add to the diagnostic accuracy as part of a biomarker panel. An examination of lung cancer patients led to the conclusion, that the use of ApoC1 serum values does not fulfill the criteria for a biomarker.

Similarly, the overexpression of ApoC1 mRNA has been analyzed in a multitude of malignancies, especially renal cancer and colorectal cancer, but also gynecological tumors, prostate cancer and pancreatic or gastric carcinoma. The conclusions remain equivocal, whether an elevated mRNA expression is associated with a better prognosis of the patient. Of note, a high mRNA expression can occur without a subsequently elevated protein

| Year | Author | Population                          | Patients | Level [µg/ml] |
|------|--------|-------------------------------------|----------|--------------|
| 1981 | Curry et al | Control                           | n.d      | 60.0         |
|      |        | Hyperlipoproteinemia                | n.d      | 132.5        |
| 1982 | Carlson and Holmquist8 | Normolipidemic men               | 29       | 63.0         |
| 1986 | Riesen and Sturzenegger2          | Apparently healthy males          | 38       | 61.0         |
|      |        | Apparently healthy females          | 32       | 65.0         |
| 1987 | Attian et al10                    | Control                           | 42       | 85.5         |
|      |        | Chronic renal failure               | 33       | 95.0         |
| 1993 | Bren et al11                      | control                           | 26       | 72.0         |
|      |        | Diabetes                            | 14       | 113.2        |
|      |        | Type V hyperlipoproteinemia         | 12       | 137.8        |
| 2003 | Cohn et al12                      | Normolipidemic                    | 89       | 105.7        |
|      |        | Hyperlipidemic                      | 88       | 128.7        |
| 2005 | Shachter et al13                  | Hispanic children                 | 362      | 62.0         |
| 2007 | Dautin et al14                    | Chronic renal failure             | 28       | 131.1        |
| 2008 | Berbee et al15                    | Sepsis                            | 17       | 13.4         |
| 2010 | Lahiry16                          | Male Oji-Cree                     | 192      | 220.7        |
|      |        | Female Oji-Cree                     | 217      | 203.9        |
|      | Xue et al17                        | Control                           | 18       | 107,000.0    |
|      |        | Pancreatico-biliary pathology       | 28       | 101,000.0    |
| 2011 | Cohen et al18                      | Control                           | 54       | 92.5         |
| 2013 | McNeal et al19                     | Vascular                          | 20       | 115.4        |
| 2014 | Ko et al20                         | Control                           | 0        | 50.0         |
|      |        | Pneumonia                          | 16       | 50.0         |
| 2018 | Al-Daghti et al21                 | Vitamin D substitution            | 120      | 35.2         |
|      | Dittrich et al22                  | control                           | 1300     | 40.0         |
| 2019 | Wang et al23                      | Control                           | 60       | 17,390.0     |
|      |        | Chronic atrophic gastritis         | 60       | 12,830.0     |
|      | Yi et al24                         | Control                           | 40       | 0.1          |
| Non-tumor patients | Number | 2943                                    | Mean   | 8584.0       |
|      |        |                                    | Median | 92.8         |
| 2010 | Xue et al17                        | Pancreatic adenocarcinoma         | 33       | 124,000.0    |
| 2011 | Cohen et al18                      | Stomach cancer                    | 101      | 31.3         |
| 2014 | Ko et al20                         | Lung cancer                       | 48       | 50.0         |
| 2019 | Wang et al23                      | Gastric cancer                    | 60       | 9530.0       |
|      | Yi et al24                         | Gastric cancer                    | 65       | 0.3          |
|      | Present study                      | glioblastoma                      | 68       | 91.9         |
| Tumor patients | Number | 375                                      | Mean   | 22,283.9     |
|      |        |                                    | Median | 71.0         |

Table 3. Overview of published ApoC1 serum levels. n.d.: not disclosed. Median values are in bold.
synthesis, the cellular subpopulations of the tumor that shed ApoC1 into the blood stream being subject to further translational control.

According to the results of the present study, namely a poor correlation of ApoC1 serum levels to tumor volume and to immunohistologic staining intensity, the subset of neuroepithelial cells does not constitute an important source of circulating ApoC1 protein. The increased detection of ApoC1 immunostaining in the vicinity of necrotic areas favors the theory of its occurrence during the activation of microglia and invading macrophages. In this way, expression of ApoC1 can be considered to be a physiological reaction of surrounding tissue and immunocompetent cells to the stimulus by the glioblastoma cells, similar to stromal reactions described in other malignant entities, and in line with increasing ApoC1 serum levels detected during recovery from sepsis.

In the cohort presented here, the stimulus and its removal were sufficient to result in detectable and significant serologic ApoC1 level changes. When comparing these values to the results from the literature, it remains questionable whether the glioblastoma-derived ApoC1 synthesis overrides the levels obtained by physiologic production in the liver or postprandial hyperlipoproteinemia (Table 3). Thus, a single absolute ApoC1 serum value does not allow conclusions concerning the activity of the glioblastoma.

As a next step, to determine whether monitoring of subsequent serum levels leads to the detection of a recurrent tumor before it becomes visible in MRI, a much larger, multicenter study with a long-time follow-up would be needed. The patients should preferentially undergo strict fasting before drawing the serum and the samples should be assessed with a high-throughput technique such as liquid chromatography mass spectrometry, allowing for the simultaneous detection of further apolipoproteins. In order to exclude hepatic, vascular or obesity-induced interferences, a normalization of values to body weight or hepatic volume might be undertaken. These settings and prerequisites then considerably interfere with the ideal of a simple and easy to use biomarker.

**Conclusion**

In the examined patients, ApoC1 serum levels fell significantly after neurosurgical resection of glioblastoma. However, no cut-off values to define tumor control or recurrence could be detected. Repeated monitoring of ApoC1 levels in a large number of individual patients, recruited in a multicenter study would be needed, to determine whether ApoC1 correlates to or is predictive for clinical symptoms and MR-imaging.

The present study yields novel and pathophysiologically most interesting information about protein shedding from glioblastoma tissue, even giving a hint on how local tumor entities might influence the systemic lipid metabolism of the host. From a practitioner's point of view, the use of serum ApoC1 levels as glioblastoma biomarker with an impact on clinical or diagnostic decision-making seems out of reach.

**Data availability**

The datasets generated and analyzed during the current study are not publicly available due to technical reasons but are available from the corresponding author on reasonable request.

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**References**

1. Groll, M. et al. MALDI-TOF mass spectrometric analysis of brain tumor cyst fluid reveals a protein peak corresponding to ApoC1 and LuzP6. Open J. Modern Neurosurg. 8, 251–263. https://doi.org/10.4236/ojmn.2018.83021 (2018).

2. Evangelou, P. et al. Assessment of ApoC1, Luz P6, C12orf75 and OCC-1 in cystic glioblastoma using MALDI-TOF mass spectrometry, immunohistochemistry and qRT-PCR. Med. Mol. Morphol. 52(4), 217–225. https://doi.org/10.1007/s00795-019-00223-8 (2019).

3. Cudaback, E. et al. Apolipoprotein C-I is an APOE genotype-dependent suppressor of glial activation. J. Neuroinflammation 9, 192. https://doi.org/10.1186/1742-2099-9-192 (2012).

4. Hambardzumyan, D., Gutmann, D. H. & Kettenmann, H. The role of microglia and macrophages in glioma maintenance and progression. Nat. Neurosci. 19(1), 20–27. https://doi.org/10.1038/nn.4185 (2016).
5. Lauer, S. J. et al. Two copies of the human apolipoprotein C-I gene are linked closely to the apolipoprotein E gene. J. Biol. Chem. 263(13), 7277–7286 (1988).

6. Hilbert, M., Kuzman, P., Mueller, W. C. & Nestler, U. The mean ApoC1 serum level in postoperative samples from neurosurgical patients is lower than in preoperative samples and during chemotherapy. Biology 11(7), 1021. https://doi.org/10.3390/biology11071021 (2022).

7. Curry, M. D., McDonathy, W. J., Fesmire, J. D. & Alaupovic, P. Quantitative determination of apolipoproteins C-I and C-II in human plasma by separate electroimmunoassays. Clin. Chem. 27(4), 543–548 (1981) (PMID: 7471419).

8. Carlson, L. A. & Holmquist, L. Concentrations of apolipoproteins B, C-I, C-II, C-III and E in sera from normal men and their relation to serum lipoprotein levels. Clin. Chim. Acta 124(2), 163–178. https://doi.org/10.1016/0009-8881(82)90384-9 (1982).

9. Riesen, W. F. & Sturzenegger, E. Enzyme-linked immunosorbent assay for apolipoprotein C-I. J. Clin. Chem. Clin. Biochem. 24(10), 723–727. https://doi.org/10.1515/ccl.1986.24.10.723 (1986).

10. Attman, P. O., Alaupovic, P. & Gustafson, A. Serum apolipoprotein profile of patients with chronic renal failure. Kidney Int. 32(3), 368–375. https://doi.org/10.1038/ki.1987.219 (1987).

11. Bren, N. D., Rastogi, A. & Kotte, B. A. Quantification of human plasma apolipoproteins C-I-C-II, and C-III by radioimmunoassays. Mayo Clin. Proc. 68(7), 657–664. https://doi.org/10.1016/0025-6196(93)90116-3 (1993).

12. Cohn, J. S. et al. Plasma concentration and lipoprotein distribution of Apo-C-I is dependent on ApoE genotype rather than the Hpa I Apo-C-I promoter polymorphism. Atherosclerosis 169(1), 63–70. https://doi.org/10.1016/s0021-9150(03)00139-4 (2003).

13. Shachter, N. S. et al. The common insertional polymorphism in the APOC1 promoter is associated with serum apolipoprotein C-I levels in Hispanic children. Atherosclerosis 179(2), 387–393. https://doi.org/10.1016/j.atherosclerosis.2004.10.032 (2005).

14. Dautin, G. et al. Hemodialysis reduces plasma apolipoprotein C-I concentration making VLDL a better substrate for lipoprotein lipase. Kidney Int. 72(7), 871–878. https://doi.org/10.1038/sj.ki.5002449 (2007).

15. Berbée, J. F. et al. Plasma apolipoprotein C1 correlates with increased survival in patients with severe sepsis. Intensive Care Med. 34(5), 907–911. https://doi.org/10.1007/s00134-008-1006-y (2008).

16. Lahiry, P. et al. APOC1 T455 polymorphism is associated with reduced obesity indices and lower plasma concentrations of leptin and apolipoprotein C-I in aboriginal Canadians. J. Lipid Res. 51(4), 843–848. https://doi.org/10.1194/jlr.P020144 (2010).

17. Xue, A. et al. Discovery of serum biomarkers for pancreatic adenocarcinoma using proteomic analysis. Br. J. Cancer 103(3), 391–400. https://doi.org/10.1038/bjc.2010.38 (2010).

18. Cohen, M. et al. Serum apolipoproteins C-I and C-III are reduced in stomach cancer patients: Results from MALDI-based peptide and immuno-based clinical assays. PLoS ONE 6(1), e14540. https://doi.org/10.1371/journal.pone.0014540 (2011).

19. McNeal, C. J. et al. Human HDL containing a novel apo-C-I isoform induces smooth muscle cell apoptosis. Cardiovasc Res. 98(1), 83–93. https://doi.org/10.1093/cvr/cvt014 (2013).

20. Ko, H. L. et al. Apolipoprotein C1 (APOCI) as a novel diagnostic and prognostic biomarker for lung cancer: A marker phase I trial. Thoracic Cancer 5(6), 500–508. https://doi.org/10.1111/1759-7714.12117 (2014).

21. Al-Daghri, N. M. et al. Sex-specific expression of apolipoprotein levels following replenishment of vitamin D. J. Steroid Biochem. Mol. Biol. 180, 129–136. https://doi.org/10.1016/j.jsbmb.2017.12.005 (2018).

22. Dittrich, J. et al. Targeted on-line SPE-LC-MS/MS assay for the quantitation of 12 apolipoproteins from human blood. Proteomics https://doi.org/10.1002/pmic.201700279 (2018).

23. Wang, M., Wang, J. & Jiang, H. Diagnostic value of apolipoprotein C-I, transthyretin and apolipoprotein C-III in gastric cancer. Oncol. Lett. 17(3), 3227–3232. https://doi.org/10.3892/ol.2019.9957 (2019).

24. Yi, J. et al. Apolipoprotein C1 (APOCI) as a novel diagnostic and prognostic biomarker for gastric cancer. Ann. Transl. Med. 7(16), 380. https://doi.org/10.21037/atm.2019.07.59 (2019).

25. Ricci, F., Kern, S. E., Hruban, R. H. & Jacobusio-Donahue, C. A. Stromal responses to carcinomas of the pancreas: Juxtapatumal gene expression conforms to the infiltrating pattern and not the biologic subtype. Cancer Biol. Ther. 4(3), 302–307. https://doi.org/10.4161/cbt.4.3.1501 (2005).

Author contributions
U.N., M.H. and J.M treated the patients, P.K. and W.C.M. performed the histologic assessments. ELISA testing, statistical work-up and writing of the draft was done by U.N. and M.H. All authors reviewed and approved the manuscript. All authors contributed to the study conception and design. Material preparation and data collection were performed by Michelle Hilbert, Ulf Nestler and Jürgen Meixensberger. Analysis was performed by Michelle Hilbert, Ulf Nestler, Peter Kuzman and Wolf C. Mueller. The first draft of the manuscript was written by Ulf Nestler and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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