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Potential for the blood-based biomarkers cytokeratin 19 fragment (CYFRA 21-1) and human epididymal protein 4 (HE4) to detect recurrence during monitoring after surgical resection of adenocarcinoma of the lung

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ABSTRACT

Objectives: The biomarkers cytokeratin 19 fragment (CYFRA 21-1) and human epididymis protein 4 (HE4) are useful in the diagnosis, prognosis, and monitoring of non-small cell lung cancer (NSCLC), but their combination has not been investigated yet. The objective of this analysis was to evaluate the ability of CYFRA 21-1 and HE4 to predict recurrence as part of follow-up monitoring in patients with adenocarcinoma (ADC) of the lung.

Materials and methods: Serum samples were collected from patients with stage I-IIIA ADC preoperatively and during follow-up at 3, 6, 12, 18, and 24 months and then every 6–12 months up to 5 years post-R0 resection. Samples were analyzed for CYFRA 21-1 and HE4 via electrochemiluminescence immunoassay. All cases of disease recurrence were verified by imaging. The diagnostic performance of CYFRA 21-1, HE4, and their combination to predict recurrence was assessed by Receiver Operating Characteristic (ROC) and corresponding area under the curve (AUC).

Results: 115 patients with ADC were included (N = 612 biomarker measurements); median age was 63 years; most had stage I–II disease (n = 97; 84.3%). All patients underwent surgical resection; 44 patients (38%) also received adjuvant chemotherapy and 16 (14%) received radiation therapy. At the median timepoint for the last blood sample collection (37 months), 31 patients (27%) had experienced recurrence. Both CYFRA 21-1 and HE4 were able to detect recurrence (AUC and 95% confidence interval [CI]): 75.9% (66.0–85.8%) and 75.4% (65.9–84.8%), respectively, but this increased with the combination (78.8% [69.0–88.6%]). At a sensitivity of 80%, the respective specificities (95% CI) for CYFRA 21-1, HE4, and the combination were 57.1% (53.0–61.2%), 57.1% (53.0–61.2%), and 69.7% (65.8–73.4%).

Conclusion: Serial measurements of serum CYFRA 21-1 and HE4 levels could provide a valuable method for follow-up monitoring of patients with ADC to detect recurrence.

Abbreviations: ADC, adenocarcinoma; AUC, area under the curve; CI, confidence interval; CT, computed tomography; CYFRA 21-1, cytokeratin 19 fragment; CXR, chest x-ray; ECLIA, electrochemiluminescence immunoassay; FDG, fluorine 18 fluordeoxyglucose; HE4, human epididymis protein 4; NSCLC, non-small cell lung cancer; PET, positron emission tomography; ROC, receiver operating characteristic; SCC, squamous cell carcinoma

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1. Introduction

Lung cancer is the primary cause of cancer deaths in both men and women, comprising 26.5% of all cases [1]. Non-small cell lung cancer (NSCLC) accounts for 85% of lung cancers, with the two major histologies being adenocarcinoma of the lung (ADC) and squamous cell carcinoma (SCC) [2].

NSCLC is only curable if caught in the early stages of the disease, where surgery is the mainstay of treatment [3,4]. However, only one in four patients are diagnosed at a potentially curable stage [5], and even in patients treated with curative intent, recurrence is common [6,7]. In a review of 1294 consecutive patients with early stage NSCLC treated with surgical resection, after a median follow-up of 35 months, 20% of patients had relapsed, predominantly within the first 2 years, and a further 7% had a second primary lung cancer [8]. Thus, in clinical practice, patients are regularly monitored for recurrence after initial treatment [9,10].

Recurrence monitoring for NSCLC is based on both a clinical exam and imaging, which can be chest x-ray (CXR), computed tomography (CT), or positron emission tomography (PET) CT scan [11–13], with CT scans used most widely in the Western world [12,14]. However, there are limitations to all of these imaging techniques, including variations in sensitivity [14] and specificity [15], high costs [16,17], variable availability, and the risks associated with repeated exposure to radiation [18–20].

There is evidence to suggest that the biomarkers cytokeratin 19 fragment (CYFRA 21-1) and human epididymis protein 4 (HE4) may be useful in the diagnosis, prognosis, and treatment monitoring of NSCLC [21–31]. These biomarker levels appear to be higher in patients with advanced disease, those with nodal and/or distant metastases, and in those with a poor performance status [27,32,33], with the majority of data regarding the prognostic utility of these biomarkers reported in the advanced disease setting [21,22,24,28–31] or across all stages of disease [25,34,35]. However, some evidence exists for CYFRA 21-1 in early-stage NSCLC [36–38], including findings from a retrospective analysis of 515 patients with NSCLC who underwent surgery, which showed that in patients with stage I disease, 3-year survival rates were lower in patients with elevated preoperative serum levels of CYFRA 21-1 than in those with normal CYFRA 21-1 levels (60.2% versus 78.4%; $p = 0.015$) [39]. Although data to support the potential utility of these biomarkers as part of treatment monitoring are currently limited, several studies have shown that reductions in CYFRA 21-1 or HE4 appear to be an early indicator of response to chemotherapy [21,22,27,29]. Among patients with early-stage NSCLC who have undergone complete surgical resection, there is also some evidence to suggest that CYFRA 21-1 may be able to predict tumor recurrence in both in SCC [40,41,43] and ADC [41,44,45]; similar finding have only been reported for HE4 in a cohort of patients with ADC [42].

Despite evidence showing the potential benefits of CYFRA 21-1 and HE4 testing in this setting, the combination of both biomarkers has not yet been investigated. Given the above, the current study investigated whether the combination of CYFRA 21-1 and HE4 could be useful as biomarkers of recurrence in ADC.

2. Materials and methods

2.1. Study design and patient population

This was a retrospective analysis of serum samples that were collected as part of a prospective longitudinal study designed to identify a molecular signature associated with poor prognosis among patients with early-stage NSCLC. In the original study, all patients were recruited between March 2006 and February 2009 at the Thoracic Clinic of the University of Heidelberg, Germany. Eligible patients were aged ≥18 years with ADC, SCC, or mixed histology, with a pathological tumor stage of pT1–2/N0 (stage I), pT3/N0 or pT1–2/N1 (stage II) or pT1–T2/N2, pT3/N1–N2 or pT4/N0–1 (stage IIIA) according to the Union for International Cancer Control (UICC) criteria, 6thedition [46]. Patients were excluded if they had a previous tumor diagnosis, if they had received neoadjuvant chemotherapy or if they had positive tumor margins following resection (≥R1). Following surgical resection, all patients received additional treatment for their disease, including adjuvant chemotherapy or radiotherapy, according to local best practice. Disease recurrences were limited to those detected in the lungs and were verified based on imaging results (CXR and CT) conducted every cycle during chemotherapy and at 3, 6, 12, 18 and 24 months, and then every 6–12 months up to 5 years post-R0 resection as part of routine follow-up monitoring. Patients with a secondary tumor detected in another location were not included in this analysis. No clinical distinction was made between detecting a true recurrence in the lung or a metachronous primary lung cancer, so the definition of recurrence included both scenarios.

The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice, and institutional review board approval was granted by the local ethics committee of the University of Heidelberg (no. 270/2001). All patients provided written informed consent for the use of their blood samples for research purposes.

2.2. Sample collection

Blood samples were collected from all enrolled patients prior to surgery (baseline), and at 3, 6, 12, 18, and 24 months, and then every 6–12 months up to 5 years post-surgery. Blood samples were stored at −80 °C within 2 h of venipuncture at the Lung Biobank (Heidelberg, Germany) – a member of the Biomaterial Bank Heidelberg (BMBH) and the Biobank platform of the German Center for Lung Research (DZL) – before being transferred to Roche Diagnostics GmbH (Penzberg, Germany) for long-term storage.

2.3. Analysis of samples (Elecsys® Method)

All samples were analyzed simultaneously for CYFRA 21-1 and HE4 concentration via electrochemiluminescence immunoassay (ECLIA) using a **cobas e**601 analyzer and Roche Elecsys® CYFRA 21-1 and HE4 assays (Roche Diagnostics GmbH, Germany) according to the manufacturer's instructions. In these sandwich assays, samples are incubated with two types of antibodies. The ECLIAs for CYFRA 21-1 and HE4 include antigen-specific biotinylated monoclonal antibodies and ruthenium complex-labeled monoclonal antibodies. Streptavidin-coated microparticles are added to the resulting sandwich complex in each assay, which becomes bound to the solid phase via the biotin-streptavidin interaction. The reaction mixture from each assay is then aspirated into a measuring cell where the microparticles are magnetically captured onto the surface of an electrode. Application of a voltage to the electrode induces a chemiluminescent emission which is measured using a photomultiplier. Results are determined via a calibration curve.

2.4. Statistical analysis

This post-hoc analysis included only samples from patients with ADC (N = 144). In addition, only patients with at least one post-surgery follow-up visit that was no later than the first recurrence and who provided a matching blood sample within ± 15 days of the recurrence date were included. Patients with a secondary tumor recurrence in another organ (e.g. prostate, bladder, colon) were excluded. For all included patients, each visit after surgery until the first recurrence was included in the analysis with exception of the following reasons: visits for which no blood sample was available, visits when the patient had severely impaired renal function (defined as estimated glomerular filtration rate [eGFR] < 15 mL/min), and visits with an inconclusive imaging result.

Mean serum CYFRA 21-1 and HE4 levels were calculated for recurrence (i.e. all samples collected from patients at the timepoint of documented recurrence) versus no recurrence (i.e. all samples collected from patients at all timepoints where they were still considered recurrence-free, irrespective of final outcome). The diagnostic performance of CYFRA 21-1, HE4, and their combination, for determining ADC recurrence was assessed using Receiver Operating Characteristic (ROC) analysis and the corresponding area under the curve (AUC) [47,48]. Assessment of the combination of CYFRA 21-1 and HE4 was based on the weighted sum of the logarithmized (base 10) markers. The weights were derived from a logistic regression model [49], which included for each visit the $\log_{10}$ of CYFRA 21-1 and HE4 as independent variables and recurrence (yes/no) as a dependent variable. The estimated coefficients from the logistic regression model were then used to form the linear combination out of the two markers:

3.87 x $\log_{10}$ (CYFRA 21-1) + 2.13 x $\log_{10}$ (HE4)

A correction for repeated measurements was not included in the logistic regression model as focus was not on the interference of model parameters but only on the derivation of reasonable estimates for the weighting factors. The diagnostic performance was assessed using the ROC/AUC in the same manner for CYFRA 21-1, HE4, and the combination. The positive predictive value (PPV) and negative predictive value (NPV) for CYFRA 21-1, HE4, and the combined score were calculated based on the observed prevalence of recurrence in this cohort (26.9%). Calculated p values of < 0.05 were considered statistically significant.

3. Results

3.1. Patient demographics

Of 144 patients with ADC enrolled in the original study, 115 met the eligibility criteria and were included in this post-hoc analysis, providing a total of 612 biomarker measurements. Reasons for patient exclusion were: no biomarker sample available (n = 8), no baseline biomarker sample available (n = 9), no biomarker sample available within ± 15 days of the recurrence date (n = 4), unclear diagnosis and/or presence of a second tumor in another organ (n = 8).

Among the remaining eligible patients with ADC, 62 (54%) were male and the median age was 64 (range 38–83 years). The majority of patients had stage I or II disease (n = 97; 84%), with the remainder having stage IIIA disease (n = 18; 16%) (Table 1).

Table 1
Patient demographics.

	All (N = 115)	Recurrence (N = 31)	No recurrence (N = 84)
Gender, n (%)			
Male	62 (54)	21 (68)	41 (49)
Female	53 (46)	10 (32)	43 (51)
Median age, years (range)	64 (38–83)	65 (44–83)	63 (38–81)
Smoking status, n (%)			
Active	26 (23)	11 (35)	15 (18)
Former	71 (62)	15 (48)	56 (67)
Never	16 (14)	5 (16)	11 (13)
Unknown	2 (2)	0	2 (2)
Stage at diagnosis, n (%)			
I	73 (63)	17 (55)	56 (67)
II	24 (21)	8 (26)	16 (19)
IIIA	18 (16)	6 (19)	12 (14)
Treatment, n (%)			
Surgery	115 (100)	31 (100)	84 (100)
Adjuvant treatment	48 (42)	18 (58)	30 (36)
Adjuvant chemotherapy	44 (38)	15 (48)	29 (34)
Adjuvant radiation	16 (14)	9 (29)	7 (8)
No adjuvant treatment	67 (58)	13 (42)	54 (64)

All patients received surgery for ADC; the vast majority had a lobectomy (n = 100; 87%); 9 patients (8%) underwent pneumonectomy, 5 (4%) underwent bi-lobectomy, and one patient (1%) had an atypical resection. Following surgery, 44 patients (38%) received adjuvant chemotherapy and 16 patients (14%) received radiation.

Patient serum samples were collected for a median of 37 months post-surgery.

3.2. Monitoring for recurrences

Median recurrence-free survival was 80.2 months. As shown in Fig. 1A, the pattern of recurrence suggested a peak between 6–9 months post-surgery. The total number of patients who experienced disease recurrence was 31 (27%). As advanced disease stage is a known risk factor for recurrence in resected NSCLC [50], as expected, recurrence rates in this study were higher among patients with stage II-IIIA (33%) versus stage I disease (23%). At the median timepoint for the last blood sample collection (37 months), 30 out of 31 (97%) cases of disease recurrence had occurred (Fig. 1**A and B**). The majority of patients with recurrent disease were male (n = 21, 68%) (Table 1).

3.3. Diagnostic performance of CYFRA 21-1and HE4 in detecting recurrence of ADC

Serum levels of both CYFRA 21-1 and HE4 were higher at baseline (i.e. prior to surgery) than at 90 days post-surgery, and also appeared to be slightly higher in the recurrence group compared with the no

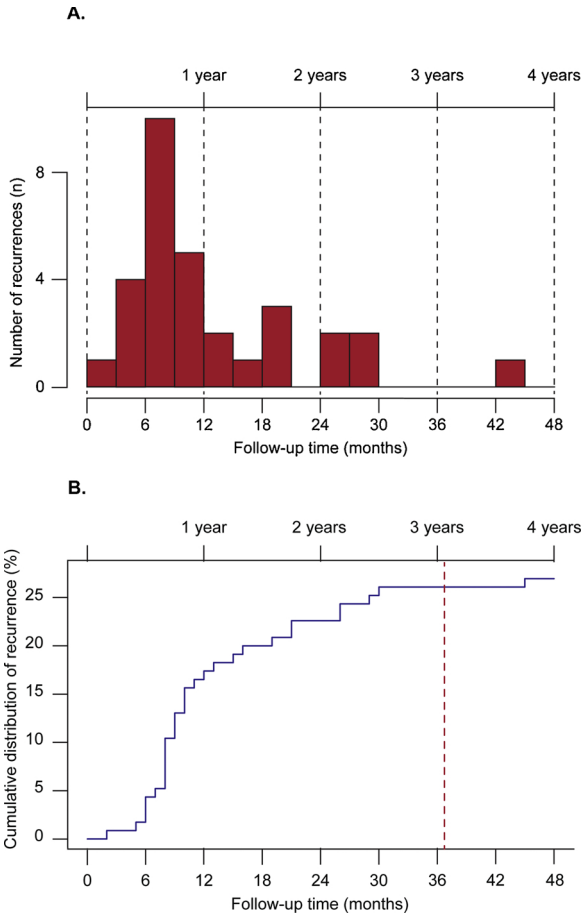

Fig. 1. Recurrence pattern over time in patients with stage I–IIIA ADC. (A) Absolute number of recurrences during each time interval (one bar denotes 3 months). (B) Cumulative incidence of recurrences over time (the dotted line denotes median duration of blood sample collection).
ADC, adenocarcinoma.

Table 2
Serum levels of CYFRA 21-1 and HE4 at baseline and 90 days post-surgery.

Tumor biomarker	Timepoint	Recurrence(N = 31)	No recurrence(N = 84)	P value**
Median CYFRA 21-1, ng/mL (range)	Baseline*	2.77 (1.16–17.25)	2.26 (0.78–18.95)	0.038
	90 days post-surgery	2.17 (0.65–4.24)	1.29 (0.66–2.74)	NS
Median HE4, pmol/mL (range)	Baseline*	113.56 (50.76–465.4)	95.99 (44.22–537.68)	0.030
	90 days post-surgery	73.18 (47.96–422.2)	71.29 (44.32–385.83)	NS

*Prior to surgery, **Wilcoxon test.
NS, not significant.

recurrence group at each timepoint, although this only reached statistical significance for baseline value comparisons (CYFRA 21-1: 2.77 vs 2.26 ng/mL, p = 0.038; HE4: 113.56 vs 95.99 pmol/mL, p = 0.030; Table 2).

In addition, among patients experiencing recurrence, serum levels of CYFRA 21-1 and HE4 were higher in samples taken at the time of recurrence compared with those derived from all samples from patients still in remission (Fig. 2). Median (range) serum levels in patients at the time of recurrence versus those from patients in remission were 2.28 ng/mL (0.78–115.61) versus 1.37 ng/mL (0.44–7.55), respectively, for CYFRA 21-1, and 105.64 pmol/mL (51.11–1500.00) versus 70.32 pmol/L (39.17–422.20), respectively, for HE4. The combined score for CYFRA 21-1 and HE4 was also higher in patients at the time of recurrence versus those from patients in remission (median [range]: -2.29 [-4.53–6.53] and -3.52 [-5.81–0.08], respectively).

The AUC measured the discriminative power of the biomarkers to distinguish patients with recurrence from those still in remission. While the AUC and corresponding 95% confidence interval (CI) of the individual biomarkers, CYFRA 21-1 (75.9% [66.0–85.8%]) and HE4 (75.4% [65.9–84.8%]), was satisfactory for detecting a recurrence, the combination of both biomarkers modestly increased the AUC to 78.8% (69.0–88.6%) (Fig. 3).

At a sensitivity of 80%, the respective specificities (95% CI) for CYFRA 21-1 and HE4 were 57.1% (53.0–61.2%) and 57.1% (53.0–61.2%) whereas the combination of both biomarkers markedly increased specificity to 69.7% (65.8–73.4%). The PPV for CYFRA 21-1, HE4, and the combined score were 8.8%, 8.8%, and 12%. The corresponding NPV were 97.9%, 97.9%, and 98.3%.

4. Discussion

Survivors of NSCLC are at risk of both disease recurrence and the development of a new metachronous primary tumor [51,52]. Currently, recurrence monitoring for NSCLC is based on clinical exam and imaging with CXR, CT, or PET-CT scan. However, there are limitations to all of these imaging techniques [11–13,53]. A recent study reported the sensitivity for CXR to detect recurrence in patients who had previously undergone lung cancer resection as only 21.2% [14]. In contrast, the sensitivity of CT scans has been reported to be as high as 100% in NSCLC patients referred for clinical evaluation of suspected recurrence, but at a cost of a low specificity of 25% [8]. Furthermore, despite the higher sensitivity and potentially earlier diagnosis of recurrence with CT scans, a convincing survival benefit for CT scans over CXRs has not been demonstrated, even in a contemporary series [54]. This was recently confirmed by the IFCT-0302 trial, which showed no survival benefit for the addition of CT and bronchoscopy to CXR as part of routine follow-up in patients with resected NSCLC [55], and so call into question the role of CT scans in NSCLC as part of routine post-surgical follow-up. Although fluorine 18 fluordeoxyglucose (FDG) PET-CT scans can be used to confirm suspicious CT findings, especially in patients who have received radiation therapy, no survival benefit has been shown for PET-CT compared with CT alone and its routine use is not recommended [56]. In addition, FDG uptake is not cancer-specific and FDG-avid areas can be seen in many benign areas, including those with tissue changes resulting from surgical procedures and chemotherapy [57]. FDG binding can also remain for up to 2 years post-scan, and histologic confirmation of recurrence is still required [57]. Furthermore, the availability of PET-CT scans is limited in some regions of the world, and the frequent use of current imaging techniques in the follow-up of potentially cured early stage NSCLC patients is confounded by cost and repeated radiation exposure [18–20]. Given this, there is a need for convenient, cost-effective, and safe alternative approaches for follow-up monitoring in these patients to reduce reliance on imaging techniques.

In our study, we hypothesized that serial measurements of CYFRA 21-1 and HE4 could facilitate detection of recurrence in patients with ADC, thereby providing a rapid, relatively cost-effective, easily accessible, and safe monitoring modality for clinicians involved in the management of these patients. Indeed, a small study (n = 50) of patients with operable, stage I–IIIA ADC showed that mean serum CYFRA 21-1 levels during post-surgical follow-up were higher among those with a recurrence versus those who remained recurrence-free [44]. Similar findings were also reported in a study of two cohorts of 48 patients each with ADC and SCC [45]. In another study of 86 patients with NSCLC (including ADC, SCC, large cell and mixed histology), all patients with high preoperative CYFRA 21-1 levels (n = 38) had CYFRA 21-1 levels within the normal range within 48 h after surgery (R0 resection). Among these, 22 patients subsequently experienced a disease recurrence, with elevated CYFRA 21-1 levels detected at the time of recurrence in all cases [41]. Unfortunately, there is little information regarding the potential role of HE4 as a predictor of recurrence in NSCLC, although one study of 104 patients with ADC showed that postoperative increases in HE4 were significantly correlated with recurrence (p = 0.032), with 5-year survival outcomes significantly lower in HE4-postive patients compared with HE4-negative patients (52.6% versus 97.1%, p = 0.001) [42]. In our study, median serum concentrations of both CYFRA 21-1 and HE4 were higher prior to surgery compared with those recorded within 90 days post-surgery. Indeed, post-surgical CYFRA 21-1 and HE4 concentrations were within the normal ranges and remained as such among patients in remission but were higher in samples taken at the time of documented recurrence.

The current analysis was restricted to patients with ADC as a preliminary analysis of data from SCC patients included in the original longitudinal study showed that there was little change in HE4 levels pre- versus post-surgery and that HE4 did not confer any additional benefit to CYFRA 21-1 in predicting recurrence in SCC (data not shown).

Collectively, these data suggest that CYFRA 21-1 and HE4 may have potential value in follow-up monitoring to predict recurrence in patients with ADC. Given this, we hypothesized that a combination of both biomarkers may provide an even more reliable indicator of recurrence. In this study, we showed that both CYFRA 21-1 and HE4 were able to detect disease recurrence with an acceptable performance, with the combination of both biomarkers modestly increasing the ability to detect recurrence (AUC 78.8%). Notably, the specificities at a sensitivity of 80% for CYFRA 21-1 and HE4 were both 57.1%, whereas the combination of both biomarkers markedly increased specificity to 69.7%, suggesting that assessment of both biomarkers could be a more reliable predictor of recurrence in this setting. We selected an 80% sensitivity cutoff as a high sensitivity was judged to be more valuable

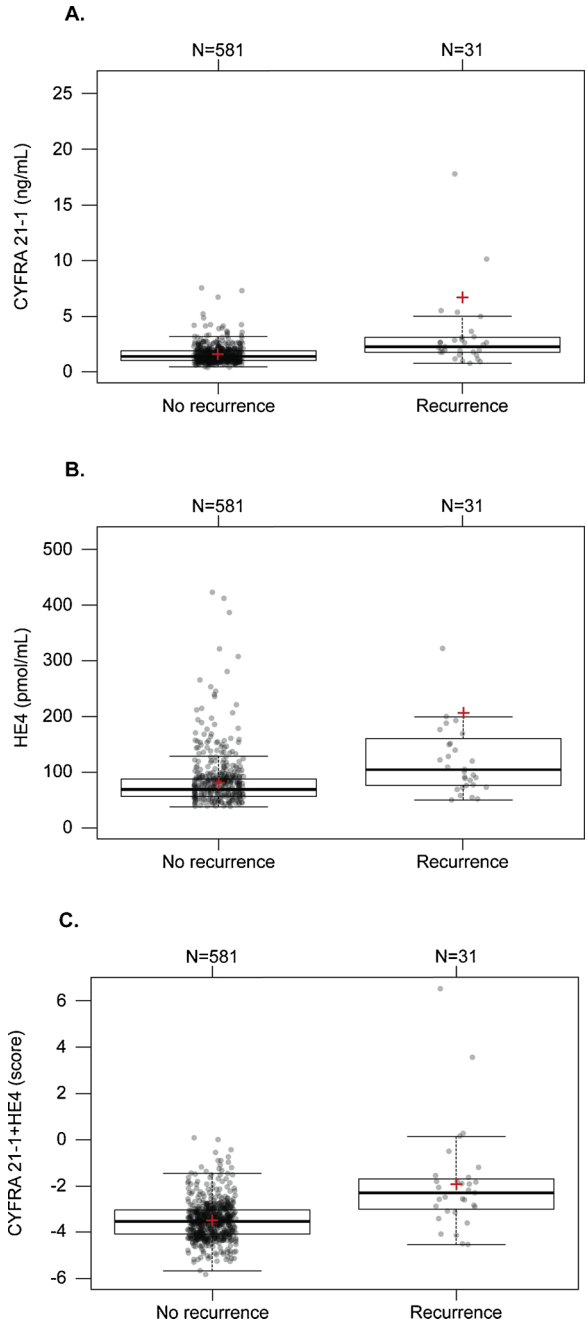

Fig. 2. Box plots of individual and median serum levels of CYFRA 21-1 (A), HE4 (B), and their combination (C) in patients with ADC experiencing no recurrence and recurrence. Median values and 1st and 3rd quartiles are depicted as box plots; crosses denote mean values. Both figures A and B are magnified for clarity and do not include results for very high values of CYFRA 21-1 (n = 1; 115.61 ng/mL) and HE4 (n = 2; both 1500 pmol/mL), all in the recurrence group.

ADC, adenocarcinoma; CYFRA 21-1, cytokeratin 19 fragment; HE4, human epididymis protein 4.

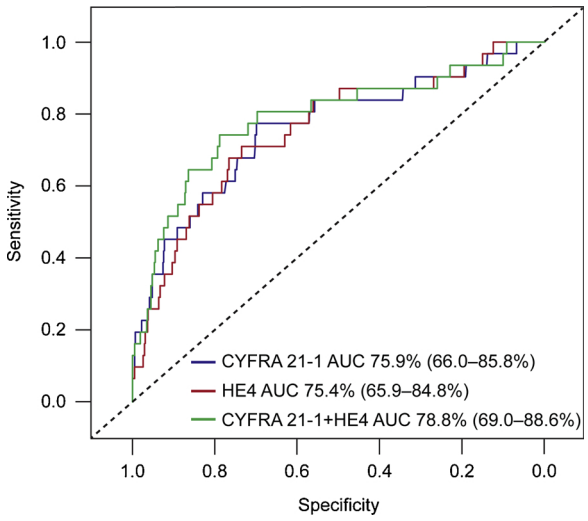

Fig. 3. Diagnostic performance of CYFRA 21-1 and HE4 for recurrence detection in ADC. AUC with 95% CI shown for CYFRA 21-1 (blue), HE4 (red), and the combination of the two biomarkers (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

ADC, adenocarcinoma; AUC, area under the curve; CI, confidence interval; CYFRA 21-1, cytokeratin 19 fragment; HE4, human epididymis protein 4.

than a high specificity for these analyses, and the limited sample size of 31 patients who experienced recurrence prevented the selection of a higher sensitivity cutoff.

Currently, there is no predefined absolute cutoff value for either CYFRA 21-1 or HE4 that can be used to define recurrence in NSCLC, although cutoff values of 2.4–3.5 ng/mL for CYFRA 21-1 [36–39] and 83.9–140 pmol/mL for HE4 [25,34] have been used in previous prognostic studies. In our study, the median serum concentration of both CYFRA 21-1 and HE4 in patients with recurrence fall within these cutoff ranges (CYFRA 21-1: 2.28 ng/mL; HE4: 105.64 pmol/mL). However, further research is still required to either define an optimal cutoff and/or provide further insights into biomarker dynamics, i.e. relative increases in biomarker levels in order to define a percentage change as a cutoff, that could be used to reliably indicate recurrence; such work would require prospective validation before it could be implemented in clinical practice. As CYFRA 21-1 may also predict recurrence in SCC [40,43] and has been proposed as a tool to facilitate the detection of distant metastases in lung cancer [33], further research in these areas is also warranted to fully elucidate the value of this biomarker in clinical practice. Taken together, our findings suggest that measuring serum CYFRA 21-1 and HE4 levels could be a helpful additional tool for follow-up monitoring in patients with early-stage ADC, which would trigger imaging if elevated. Moreover, the high NPVs reported in our study suggest that regular assessment of these biomarkers could provide ongoing assurance to both the physician and patient that there is no disease recurrence, which may be particularly useful in situations where regular imaging is problematic due to long waiting times, costs, or travelling distance for the patient.

Although not assessed in our study, dynamic monitoring of CYFRA 21-1 and HE4 (e.g. repeat testing 4 weeks after an abnormal level) could potentially improve the test performance, as shown with other solid tumors [58,59], without substantially increasing cost. Moreover, given the frequency of recurrence in NSCLC [6,7], the need for patients to be closely monitored after initial treatment [9,10], and the limitations of current follow-up imaging techniques outlined earlier [14–20], a biomarker-based monitoring approach could be particularly useful in this setting.

Thus, although further research is needed, the results presented here suggest that regular CYFRA 21-1 and HE4 testing after surgical resection may provide clinicians with sufficient information to inform their clinical assessments during follow-up monitoring of patients with early-stage NSCLC. If validated, CYFRA 21-1 and HE4 could then be used as part of routine follow-up monitoring and would reduce or remove the need for regular follow-up scans as long as biomarker levels remain low, thereby reducing costs, patient inconvenience, and exposure to radiation. Crucially, this follow-up procedure could also allow clinicians to expedite investigations and potential treatment for recurrence

in cases of elevated biomarkers, without waiting for scheduled 6- or 12-monthly follow-up scans.

The main limitations of this study are the retrospective nature of the biomarker analysis and the relatively small sample size from a single cancer center. As treatment following surgical resection was not stipulated in the original prospective study, samples used in this analysis were also from a heterogeneous study population who were treated with a range of different therapy regimens. Nevertheless, the results described here are promising and warrant further evaluation and validation as part of a prospective analysis in order to define the clinical utility of CYFRA 21-1 and HE4 for follow-up monitoring and recurrence detection in patients with ADC.

5. Conclusions

This novel algorithm of serial serum measurements of CYFRA 21-1 and HE4 could provide a valuable method for follow-up monitoring and recurrence detection in patients with early-stage ADC after initial treatment with curative intent.

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Conflict of interest

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Patents

Patent WO 2017/103034 containing data included in this manuscript is currently pending.

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