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Detection of circulating tumor cells as a prognostic factor in patients undergoing radical surgery for non-small-cell lung carcinoma: comparison of the efficacy of the CellSearch Assay™ and the isolation by size of epithelial tumor cell method

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Comparison of the efficacy of different enrichment methods for detection of circulating tumor cells (CTCs) before radical surgery is lacking in non-small-cell lung carcinoma (NSCLC) patients. Detection and enumeration of CTCs in 210 consecutive patients undergoing radical surgery for NSCLC were evaluated with the CellSearch Assay™ (CS), using the CellSearch Epithelial Cell Kit, and by the isolation by size of epithelial tumor (ISET) method, using double immunolabeling with anti-cytokeratin and anti-vimentin antibodies. CTCs were detected in 144 of 210 (69%) patients using CS and/or ISET and in 104 of 210 (50%) and 82 of 210 (39%) patients using ISET and CS, respectively. Using ISET, 23 of 210 (11%) patients had vimentin-positive cells with cytological criteria of malignancy. Disease-free survival (DFS) was worse for patients with CTCs compared to patients without CTCs detected by CS alone ($p < 0.0001$; log rank = 30.59) or by ISET alone ($p < 0.0001$; log rank = 33.07). The presence of CTCs detected by both CS and ISET correlated even better with shorter DFS at a univariate ($p < 0.0001$; log rank = 42.15) and multivariate level (HR, 1.235; 95% CI, 1.056–1.482; $p < 0.001$). CS and ISET are complementary methods for detection of CTCs in preoperative radical surgery for NSCLC. CTC detection in resectable NSCLC patients using CS and/or ISET could be a prognostic biomarker of great interest and may open up new avenues into improved therapeutic strategies for lung carcinoma patients.

Despite the different therapeutic strategies developed to date, non-small-cell lung carcinomas (NSCLC) have poor prognosis, because overall survival after 5 years is 20–25% for all stages.[1–7] The main cause of death of NSCLC results from distant metastases. It is noteworthy that a subpopulation of patients with early-stage NSCLC, completely resected by surgery, rapidly develops metastasis. This indicates that occult micrometastases, not detectable even with high-resolution imaging procedures, can be present before surgery.[8] These micrometastases may be initiated by circulating tumor cells (CTCs) present in the peripheral blood, which detach from the primary tumor at an early stage.[9] Thus, detection of CTCs before surgery might improve the treatment and the prognosis of patients with resectable NSCLC.

Different methods to detect CTCs in patients with carcinoma have been developed.[10,11] Previous studies have used indirect molecular methods such as reverse transcription-polymerase chain reaction (RT-PCR), quantitative RT-PCR (qRT-PCR) and nested RT-PCR or have used indirect immunomediated methods using immunolabeling of cells enriched by different approaches including immunomagnetic separation with magnetic beads coated with epithelial-specific antibodies (BerEP4, EpCAM), laser scanning cytometry and, more recently, a microfluidic device (the CTC chip).[10–12] qRT-PCR- and nested RT-PCR-based methods analyze the expression of a given transcript marker, compared to a reference marker expressed in any cell, which would be indicative of the presence of tumor cells. Moreover, the main advantage

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Table 1. Clinical and pathological characteristics of patients undergoing surgery for resectable non-small-cell lung carcinoma

Variables	Overall[1]
Patient cohort	210
Age (years)	
Mean	63
Range	33–82
Gender	
Male	152 (72)
Female	58 (23)
Smoking status	
Never smoked	24 (12)
Former or current smokers	186 (88)
Mean (range) P/Y	46 (1–75)
Tumor size (cm)	
Mean	3.8
Range	0.4–17
Histologic cell type	
Adenocarcinoma	120 (57)
Squamous cell carcinoma	57 (27)
Neuroendocrine carcinoma	7 (3)
Large cell carcinoma	9 (5)
Adenosquamous carcinoma	2 (1)
Sarcomatoid carcinoma	10 (5)
NSCLC (NOS)	5 (2)
pTNM stage	
I	91 (43)
II	40 (19)
III	60 (29)
IV	19 (9)

[1]Values are expressed as *n* (%).
Abbreviations: TNM: tumor node metastasis; NSCLC: non-small-cell lung carcinoma; NOS: not otherwise specified.

of these methods is the higher sensitivity in comparison with the reported sensitivity of immune-mediated detection.[11] Conversely, direct methods contribute to diagnostic identification of CTCs. Given the important constraints of immune labeling and RT-PCR assays, direct diagnosis of CTCs can only be obtained by cytopathological analysis of the isolated cells and/or by the analysis of their genome, which provides clues to the neoplasmic nature of the cell.[11] Currently, direct methods are more rarely used for CTC detection.[13–15]

These indirect and direct methods have been used to detect CTCs before, during and after surgery.[16–18] Some of these methods allow detection of the presence of biomarkers.[12,19] Most of the indirect methods have been used to detect CTCs in patients with NSCLC.[12,20–28] However, there is a considerable variability in the numbers of positive samples when using these direct and indirect techniques. Moreover, the lack of standardization of these different detection methods can act as a powerful restraint to effective implementation of CTC measurement in clinical routine practice.

Among the different indirect methods for CTC detection, the CellSearch Assay™ (CS) is currently used to detect CTCs.[9] This method is approved by the Food and Drug Administration in the United States for the follow-up of patients with breast, colon and prostate metastatic carcinomas.[9] CS is an EpCAM-based method for enrichment of CTCs in blood patients.[9] This method has been used to detect CTCs in metastatic NSCLC[29] and, more recently, in primary NSCLC before surgery.[30] CS is certainly one of the most sensitive indirect methods described for CTCs detection.[11] However, the specificity of CS has been recently disputed.[31–33] In particular, EpCAM expression of CTCs can be downregulated and, therefore, CS may miss detection of some CTCs.[31,33] Among the direct methods used to detect CTCs, the isolation by size of epithelial tumor (ISET) cell technology allows substantial enrichment of circulating epithelial cells.[14,15] Moreover, because cells are available for cytopathological analysis, the method should provide specificity and allow development of an immunocytochemical approach for phenotype identification of CTCs. However, to date, this method has not been used to detect CTCs in NSCLC patients.

Currently, there is a lack of data concerning the comparison of indirect and direct methods for detection of CTCs from the same cohort of patients undergoing radical surgery for NSCLC. Our study was designed to compare the efficacy of CS and ISET technologies to detect CTCs in blood samples taken from the same cohort of 210 patients with resectable NSCLC. In addition, each method used to detect CTCs was evaluated to correlate the presence of CTCs and the prognostic value.

Material and Methods

Patients

Two hundred and ten consecutive patients who underwent surgery for NSCLC (including 18 patients with neoadjuvant chemotherapy) between December 2007 and November 2010 at the Pasteur Hospital (Department of Thoracic Surgery, CHU of Nice, France) were included in our study. The patients received the necessary information concerning the study, and consent was obtained from each of them. The study was approved by the local ethics committees (CHU of Nice). The main clinical and pathological data are summarized in Table 1. Briefly, there were 152 (72%) men and 58 (23%) women (mean age 63 years; range 33–62 years). Eighty-eight percent of the patients were smokers (average 46 P/Y; range 1–75 PY). Patients had no transbronchial and/or transparietal chest biopsies at least 15 days before surgery. Table 1 shows morphological classification and tumor staging after surgical excision according to WHO criteria[34] and the international tumor-node-metastasis system.[5] The median follow-up at the time of analysis was 15 months (1–28

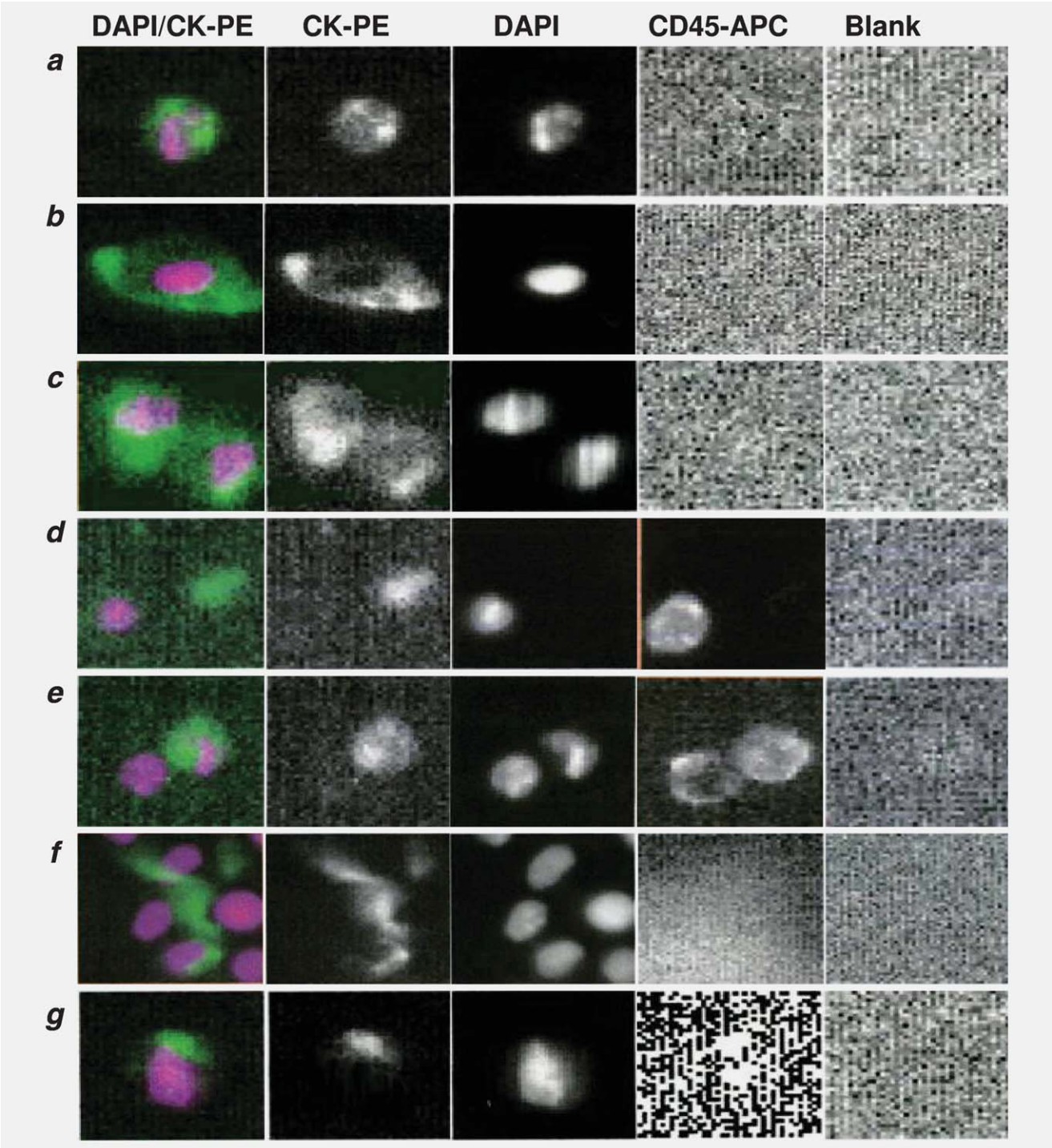

Figure 1. Gallery of circulating tumor cells (CTCs) from the CellSpotter Analyzer obtained from 7 ml of blood from patients with resectable non-small-cell lung carcinoma. Cells captured with an antiepithelial cell adhesion molecule (Ep-CAM) antibody were stained with 4′,6-diamino-2-phenylindole (DAPI), with an anti-cytokeratin antibody conjugated with phycoerythrin (CK-PE) and with an anti-CD45 antibody conjugated with allophycocyanin (CD45-APC). (*a*, *b*) Cells with a round (*a*) to oval (*b*) morphology, a visible DAPI-positive nucleus, positive CK-PE staining in the cytoplasm and negative staining for CD45-APC were considered as typical intact CTC. (*c*) Examples of intact CTC present as clusters that are observed less frequently. (*d*) Contaminating leukocytes were identified as DAPI+/CK−/CD45+ cells. (*e*) The CD45-positive cells were not considered as CTC even when cells were positively stained for DAPI and CK-PE. (*f*, *g*) Images of cells not included in the CTC count, but frequently observed in the CTC analysis of resectable NSCLC patients. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

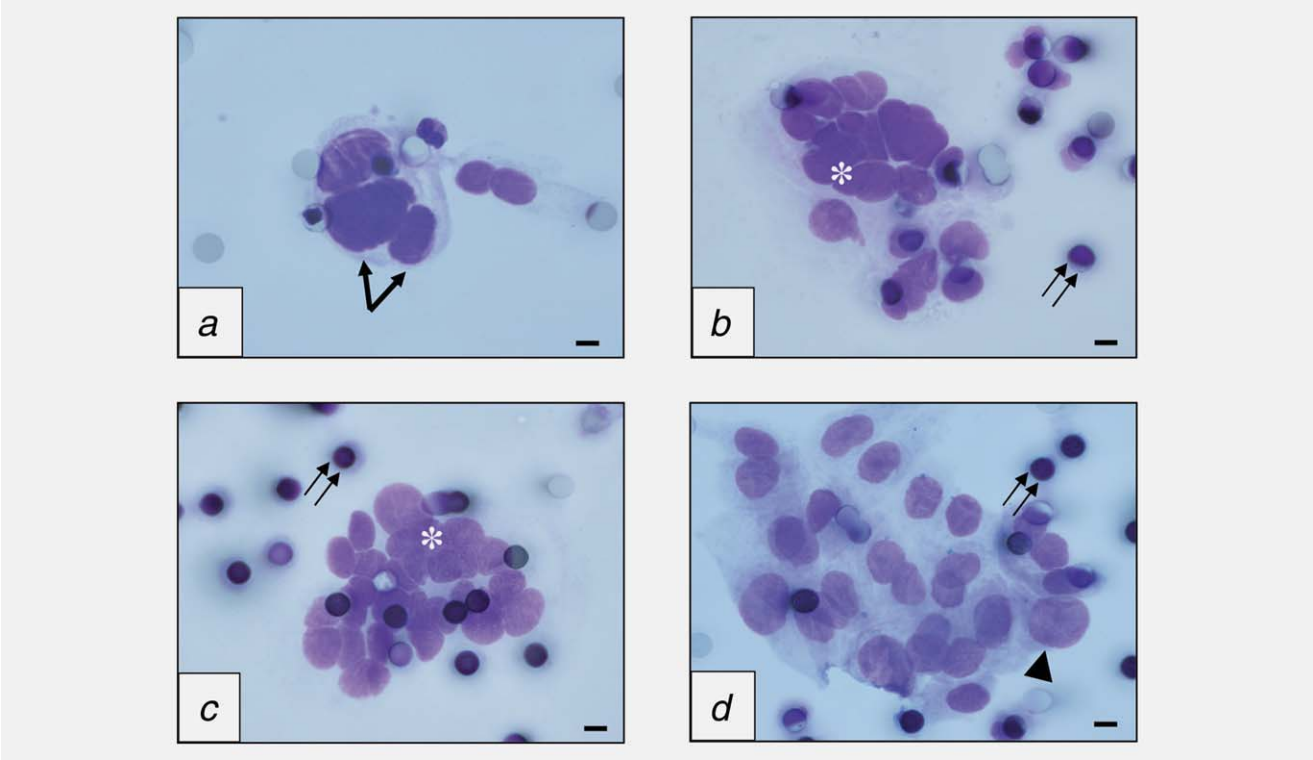

Figure 2. Cytomorphological analysis of circulating tumor cells (CTCs) detected on filtered blood using the ISET method in patients with resectable non-small-cell lung carcinoma. (*a–d*) Cells showing cytological malignant features isolated preoperatively by the ISET method in patients with a resectable lung adenocarcinoma (*a*), squamous cell carcinoma (*b*), large cell carcinoma (*c*) and sarcomatoid carcinoma (*d*). (*a–d*) Original magnification ×1,000; MGG staining; bars: 8 μm (arrows: anisonucleosis; arrows head: irregularity and large nuclei; asterisks: tridimensional sheets; double arrows: pores of the filter). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

months) according to the method of Schemper and Smith.[35] The sensitivity of CTC detection was used to determine disease-free survival (DFS). A control group consisted of 40 healthy donors without previous history of cancer. There were 30 (75%) men and 10 (25%) women, all smokers (average 15 P/Y; range 2–25 PY).

Methods

CS and ISET technologies were carried out using previously described methods with slight modification.[11] Two consecutive blood samples were collected from each individual to serve for the two methods. The first 4 ml of peripheral blood was discarded to avoid contamination with cytokeratin-positive skin cells. For CS, 7 ml of peripheral blood was taken before anesthesia and collected in the CellSave preservative tube (Veridex LLC, Raritan, NJ). Samples were maintained at room temperature and processed within 72 hr of blood collection. Briefly, the CS system (Veridex) consists of a CellPrep system, the CellSearch Epithelial Cell Kit and the CellSpotter Analyzer. The CellPrep System is a semiautomated sample preparation system, and the CellSearch Epithelial Cell Kit consists of ferrofluids coated with epithelial cell-specific EpCAM antibodies to immunomagnetically enrich epithelial cells. In the final processing step, the cells are resuspended in the MagNest Cell Presentation Device (Veridex). This device consists of a chamber and two magnets that orient the immunomagnetically labeled cells for analysis using the CellSpotter Analyzer. The criteria for definition of a CTC include a round to oval morphology, a visible nucleus (DAPI positive), positive staining for cytokeratin in the cytoplasm and negative staining for CD45 (Fig. 1). CTC enumeration was expressed as the number of positive cells per 7 ml of blood.

For the ISET method, Métagenex, Paris, France, 10 ml of peripheral blood was collected in parallel in buffered EDTA, maintained at 4°C and processed within 1 hr of blood collection. The filtration module contains ten wells, making it possible to load and filter ten individual samples in parallel. Briefly, after blood filtration, the membrane is gently washed with phosphate-buffered saline (PBS) (Sigma, Paris, France), disassembled from the filtration module and allowed to air dry. The membrane was cut into two parts containing, respectively, seven spots for immunocytochemistry and three spots for May Grünwald Giemsa (MGG) staining for further cytological analysis. Immunocytochemistry was performed using double immunolabeling with a pan-cytokeratin antibody (mouse, clone KL-1, Immunotech, Marseille, France, diluted at 1:100), which recognizes cytokeratins 2, 5, 6, 8, 10, 11, 14/15, 18 and 19, and an anti-vimentin (mouse, clone

Table 2. Analysis of the correlation between the presence of CTCs detected by isolation by size of epithelial tumor (ISET) and CellSearch assay (CS) methods

		CellSearch		
Method		+	−	Total
ISET	+	42	62	104
	−	40	66	106
Total		82	128	210

p value = 0.7 (χ^2 test). Kappa index of agreement = 0.02.

V9, Dako, Paris, France, diluted at 1:200) antibody applied to filters for 45 min at room temperature. Filters were then washed three times with PBS at room temperature, pH 7.4, and incubated for 45 min with secondary anti-phosphatase and anti-peroxidase antibodies (Dako), respectively. Immunostained and MGG-stained spots were then examined by light microscopy using different steps: (*i*) observation at 100× and 200× original magnification to look for CTCs and to count these cells, and (*ii*) observation at 630× and 1,000× original magnification with oil immersion for detailed cytomorphological analysis. The following criteria were taken into account to characterize the detected nonhematological cells: irregularity and size of the nucleus, anisonucleosis, nuclear hyperchromatism, nucleocytoplasmic ratio, size and number of nucleoli and presence of tridimensional sheets. CTCs were characterized by the presence of at least three of the following criteria: anisonucleosis (ratio > 0.5), nuclei larger than three calibrated pore size of the membrane (8 μm) (>24 μm), irregular nuclei and presence of three-dimensional sheets (Fig. 2). The results of samples from 250 individuals (210 patients and 40 healthy individuals) entered into the study and processed by CS and ISET were analyzed by four operators (ES, CL, VL and VH) working blindly, without knowledge of the clinical and pathological characteristics of the patients.

A CTC was defined as a cell with an epithelial antigen detected with the CellSearch Epithelial Cell Kit. The cutoff was one detected cell per CS. When using ISET, a CTC was defined as a cell with epithelial and/or vimentin antigens detected by immunocytochemistry and showing cytological features of nonhematological circulating cells. When using the ISET method, only CTC evaluated by IHC was counted (to consider the number of CTC in the same volume of blood, *i.e.*, 7 ml for the CS and ISET methods).

Statistical analysis

The χ^2 analysis was used to evaluate whether CTCs detected by ISET correlated with CTCs detected by CS. The Cohen's kappa coefficient was used to assess the agreement between these methods. In addition, the χ^2 analysis was used to explore the association between CTCs detected by ISET and CS methods and the histological subtype and pTNM stage. Survival time was calculated using Kaplan–Meier estimates, and differences between survival curves were analyzed by means of the log rank test. Multivariate Cox analyses were carried out to examine whether the presence of CTC, detected either by ISET or CS method or by both methods, was an independent prognostic factor for DFS with adjustment for relevant clinicopathological covariates. Variables that were associated with survival with a p value <0.20 in the univariate analysis were included in a multivariate regression. The variables included in the model for DFS were pTNM stage and histology. Differences were considered significant when a p value was less than 0.05. All statistical

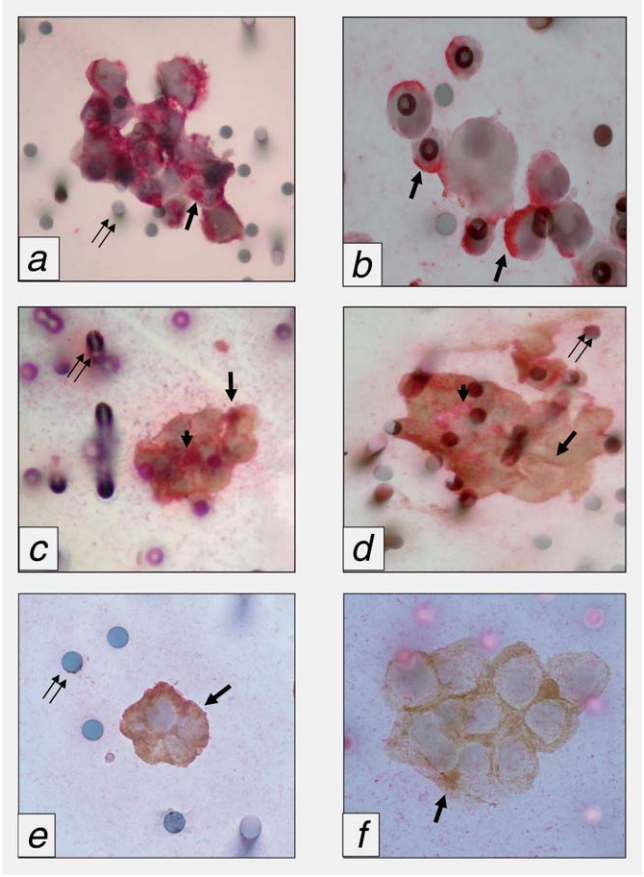

Figure 3. Clusters of immunostained circulating tumor cells (CTCs) observed on filtered blood using the ISET method in patients with resectable non-small-cell lung carcinoma. (*a*, *b*) CTCs expressing only the pan-cytokeratin antigen in patients with lung adenocarcinoma (*a*) and squamous cell carcinoma (*b*) (arrows, CTCs). (*c*, *d*) CTCs coexpressing pan-cytokeratin and vimentin antigens in patients with lung adenocarcinoma (*c*) and squamous cell carcinoma (*d*) (arrowheads: pan-cytokeratin expression; arrows, vimentin expression). (*e*, *f*) CTCs expressing only the vimentin antigen in patients with lung adenocarcinoma (*e*) and squamous cell carcinoma (*f*) (arrows: CTCs). (*a*–*f*) Original magnification ×1,000; bars: 8 μm; immunophosphatase staining with a pan-cytokeratin antibody (KL1) and immunoperoxidase staining with an anti-vimentin antibody (double arrows: pores of the filters). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 3. Presence of CTCs detected by the CellSearch assay and isolation by size of epithelial tumor (ISET) methods according to pTNM staging and histological classification

Variables	ISET+	*p* value*	Cell Search +	*p* value*	ISET+ and Cell Search+	*p* value*	ISET+ or Cell Search+	*p* value*
Histologic cell type								
Adenocarcinoma	64 (53%)	0.74	51(43%)	0.3	27 (23%)	0.23	88 (73%)	0.066
Squamous cell carcinoma	23 (40%		18 (32%)		7 (12%)		34 (60%)	
Neuroendocrine carcinoma	4 (57%)		2 (29%)		1 (14%)		5 (71%)	
Large cell carcinoma	5 (56%)		4 (44%)		2 (22%)		7 (78%)	
Adenosquamous carcinoma	1 (50%)		0 (0%)		0 (0%)		1 (50%)	
Sarcomatoid carcinoma	4 (40%)		3 (30%)		2 (20%)		5 (50%)	
NSCLC (NOS)	3 (60%)		4 (80%)		3 (60%)		4 (80%)	
pTNM stage								
I	44 (48%)	0.50	33 (36%)	0.8	13 (14%)	0.07	64 (70%)	0.59
II	24 (60%)		18 (45%)		12 (30%)		30 (75%)	
III	27 (45%)		24 (40%)		13 (22%)		38 (63%)	
IV	9 (47%)		7 (37%)		4 (21%)		12 (63%)	

*p value significant at the 0.05 level.
Abbreviations: TNM: tumor node metastasis; NSCLC: non-small-cell lung carcinoma; NOS: not otherwise specified.

evaluations were performed using the SPSS for Windows software system, version 11.0 (SPSS, Chicago, IL).

Results

CTCs were detected in 144 of 210 (69%) patients using the CS and/or ISET method. Moreover, CTCs were detected in 104 of 210 (50%) patients using the ISET method, independently of the CS method, and in 82 of 210 (39%) patients using the CS method, independently of the ISET method, respectively ($p = 0.03$) (Table 2). CTCs were detected in 44 (21%) patients when a cutoff of two cells was considered for CS analysis (not shown). CTCs were detected in 62 of 210 (30%) patients when using the ISET method and not detected by CS and in 40 of 210 (19%) patients when using the CS method and not detected by ISET, respectively ($p = 0.01$) (Table 2). Only 42 of 210 patients (20%) showed CTCs detected both by the CS and ISET methods (Table 2). No correlation was found between CTCs detected by ISET and CS methods ($p = 0.7$; $\kappa = 0.02$), indicating that measurements assessed by these two methods are independent (Table 2). Using ISET, the majority of CTCs exhibited malignant cytopathological criteria on MGG staining (Fig. 2). Moreover, similar morphological features were noted for CTCs isolated from patients with adenocarcinoma and squamous cell carcinoma as well as other rare histological subtypes of NSCLC (Fig. 2). CTCs were isolated or grouped in sheets having between 3 and >100 CTCs (Fig. 2). Corresponding immunostained cells expressed cytokeratin alone in 27 of 210 (13%) cases or in association with vimentin in 55 of 210 cases (26%) (Fig. 3). However, in 23 of 210 (11%) patients, CTCs were only positive for vimentin (Fig. 3). These latter cells showed cytological malignant features. Moreover, the expression of TTF1 was not detected in a majority of CTCs, whereas the same primary lung adenocarcinomas expressed TTF1 (data not shown).

The number of CTCs detected by CS varied from 1 to 23 cells (mean: 12 cells), whereas the number of CTCs detected by ISET varied from 1 to 150 cells (mean: 34 cells), ($p < 0.01$; data not shown). The presence of CTCs detected by these two methods was independent of disease staging and of the histology subtypes of carcinomas (Table 3). No CTCs were detected in control individuals using the ISET and CS methods.

Patients without CTCs had a significantly longer DFS compared to patients with CTCs detected by CS alone ($p < 0.0001$; log rank = 30.59) (Fig. 4*a*) or ISET ($p < 0.0001$; log rank test = 33.07) (Fig. 4*b*). This significance was even higher in patients without CTCs compared to patients with CTCs detected by CS and/or ISET ($p < 0.0001$; log rank = 42.15) (Fig. 4*c*). Subsequently, the presence of CTCs as detected by the CS (HR, 1.564; 95% CI, 1.264–4.673; $p = 0.008$) or ISET (HR, 1.372; 95% CI, 1.123–3.286; $p = 0.006$) methods or by both methods (HR, 1.235; 95% CI, 1.056–1.482; $p < 0.001$) was a significantly independent prognostic factor for shorter DFS, as demonstrated by the multivariate survival analysis using the Cox's regression model (Table 4).

Discussion

Our study demonstrates that CS and ISET technologies can detect preoperative CTCs in patients with resectable NSCLC. CS and ISET present similar sensitivities for detection of CTCs, if we consider that the detection of CTCs is only based on epithelial antigen detection. Interestingly, the average number of CTCs detected by ISET in comparison to CS

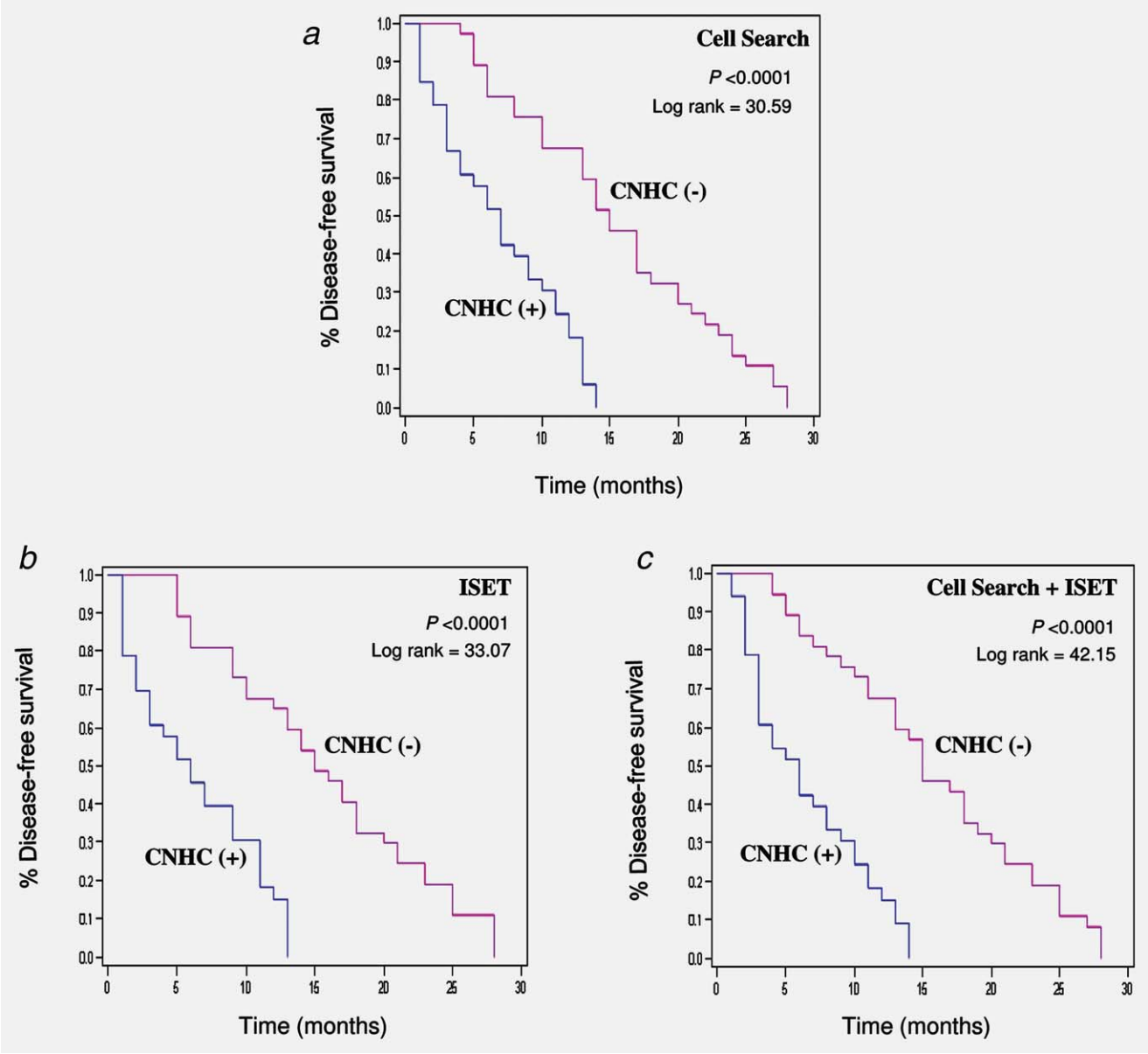

Figure 4. Kaplan–Meier curves of disease-free survival duration stratified according to the presence (+) and the absence (–) of circulating nonhematological cells (CNHC) detected by CellSearch (CS) (*a*) or isolation by size of epithelial tumor (ISET) (*b*) and by CS and ISET (*c*) methodology. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

was higher. Moreover, we observed that the number of patients with detected CTCs was higher when the two methods were used in parallel compared to each method used independently. Finally, it is noteworthy that following double immunostaining of filters after ISET, we observed in a subpopulation of patients without CTCs detected by CS, some circulating cells with nonhematological features, but with cytological malignant criteria, which expressed only vimentin.

In a previous study, 34 of 168 (20%) patients with metastatic NSCLC showed two or more CTCs detected by CS.[29] Moreover, in our study, 14, 10 and 6% of these patients demonstrated more than 5, 10 and 50 CTCs, respectively.[29] In another study using CS, CTCs were detected in peripheral blood in 5 of 30 (16.7%) patients undergoing surgery for NSCLC.[30] Interestingly, a good correlation of the enumeration of CTCs with the radiographic response following first-line chemotherapy administered in NSCLC patients was demonstrated in a recent study.[36] Using CS, we found in our series a high percentage of NSCLC patients with detection of at least one CTC (39%). However, when the cutoff was at least two CTCs, this percentage was lower (21%).

ISET technology is a direct method that allows cytomorphological analysis of CTCs.[11] For this reason, it offers a number of advantages, particularly for immunocytochemical

Table 4. Multivariate Cox proportional hazard regression analysis of predicting factors for disease-free survival in 210 resectable NSCLC patients

Prognostic factor	HR	95% CI	*p* value*
Histology			
Squamous cell carcinoma	2.764	1.223–5.342	0.023
Other subtypes	1		
pTNM stage			
I + II	0.289	0.108–0.772	0.016
III + IV	1		
CellSearch method			
CNHC+	1.564	1.264–4.673	0.008
CNHC−			
ISET method			
CNHC+	1.372	1.123–3.286	0.006
CNHC−	1		
CellSearch + ISET methods			
CNHC+	1.235	1.056–1.482	<0.001
CNHC−			

*p value significant at the 0.05 level.
Abbreviations: HR: hazard ratio; CI: confidence interval.

examination using different antibodies to better characterize the CTCs. However, some CTCs may loss a certain number of antigens normally expressed in the primary tumor. Thus, we failed to detect the expression of TTF1 in a majority of CTCs, whereas the same patients had TTF1-positive lung adenocarcinomas (data not shown). This can be explained by epithelial–mesenchymal transition (EMT), during which adenocarcinoma cells can downregulate TTF1 expression.[37] Similarly, some CTCs may have weak or no cytokeratin expression because cell dedifferentiation inducing loss of these antigens can be present during EMT. This is in keeping with our observation of a couple of circulating cells with nonhematological features that express only vimentin. We, therefore, speculate that these cells are indeed CTCs, because they demonstrate cytological malignant criteria. Moreover, it is possible that only cells coexpressing cytokeratin and vimentin or expressing vimentin alone may have a more malignant invasive potential. Further studies using molecular biology tools are needed to better characterize these potentially different subpopulations of cells and to confirm their malignant potential. Recently, genetic abnormalities identified by an antigen-independent FISH-based assay for CTCs have been suggested as a diagnostic and prognostic feature in patients with NSCLC.[38] Moreover, CTCs in combination with serological cell death assays may be used as pharmacodynamic biomarkers underlying the disease response in patients with small-cell lung cancer undergoing standard chemotherapy.[39]

There are limitations in performing a comparison of the CS and ISET methods. In the literature, CTCs have been defined as cells detected in peripheral blood that express cytokeratin antigens. In particular, all cells positively isolated by the CS system with anti-EpCAM antibodies have been called "CTCs." This can overestimate the real number of malignant cells present in the peripheral blood, because some monocytes can express cytokeratins and a subpopulation of epithelial cells might be nonmalignant.[11] Conversely, CTCs can be underestimated when using only the CS method. Thus, some CTCs probably express only vimentin because EMT allows migration of tumor cells through the stroma, to cross the endothelial barrier, and to circulate in the peripheral blood.[11] Consequently, CTCs that express only vimentin cannot be detected by CS. Thus, in our study, a couple of patients had CTCs expressing only vimentin when detected by ISET, whereas in the same population no CTCs were isolated by CS. However, we cannot definitively assume that all of these latter cells are "CTCs," even though the cytomorphological analysis of most of these cells showed malignant features. Further studies are needed to better characterize these cells using a panel of antibodies raised against different antigens. Finally, some of these cells may correspond to other nonhematological circulating cells, in particular endothelial cells.[40,41]

It is noteworthy that, in our work, the presence of CTCs detected by CS and/or ISET methods was associated with a pejorative outcome. The latter results demonstrated that preoperative CTCs detection in resectable NSCLC patients can be a prognostic biomarker in lung oncology practice.

In conclusion, CTCs can be detected by CS and ISET in NSCLC patients undergoing radical surgery. Only a fraction of this population had simultaneous detection of CTCs by ISET and CS, but the percentage of patients with detected CTCs is higher when combining the two methods. This result underlies the complementarity of the two methods for detecting CTCs preoperatively. CS failed to detect CTCs expressing only vimentin, which can be observed when using ISET.

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