Serum CYFRA 21-1 in Advanced Stage Non-Small Cell Lung Cancer: An Early Measure of Response

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ABSTRACT

Objectives: **Our objective was to test the prognostic importance of both the pretreatment level and change in serum CYFRA 21-1 after one cycle of chemotherapy in patients with advanced non-small cell lung cancer (NSCLC) and to compare these two CYFRA variables to routine clinical stage and response as measured by imaging.**

Patients and Methods: **Our patients consisted of 58 with advanced NSCLC who were treated with chemotherapy. Fourteen were stage IIIa, 8 stage IIIb, and 36 stage IV, and none had received previous treatment. The choice of chemotherapy was left to the discretion of the treating physicians. We collected two serum samples, one before the first cycle of chemotherapy and the second before the second cycle, and analyzed these for serum CYFRA 21-1 using an electrochemiluminescence immunoassay and the ElecSys 2010 system (Roche Diagnostics Corp., Indianapolis, IN). We expressed changes in CYFRA in terms of the natural ratio logarithm of post-treatment to pretreatment CYFRA, and we used the Cox proportional hazards model to analyze survival time.**

Results: **Patients experienced an average drop of 27% in serum CYFRA after the first cycle of chemotherapy. Furthermore, the Cox model demonstrated that both the initial natural logarithm of serum CYFRA and presence of >27% drop in CYFRA were significantly related to subsequent survival (model $P < 0.0006$), but neither clinical stage nor clinical response related to survival ($P > 0.1$).**

Conclusion: **In advanced stage NSCLC, the initial level of serum CYFRA appears to provide more prognostic information than clinical stage. Furthermore, a drop of >27% in CYFRA after one cycle of therapy adds prognostic information, so that this threshold appears to be an early measure of response to chemotherapy.**

INTRODUCTION

In general, measures of response to chemotherapy for advanced stage NSCLC[2] have not been optimized, *e.g.*, approximately one-third are said to respond to chemotherapy, and yet Johnson *et al.* (1) have warned that the impact of higher response rates on survival in such patients is at best modest. Clearly, it does little good to show that a therapy improves response unless that response translates into longer survival. Furthermore, traditional measures of tumor response in advanced stage NSCLC require ≥6-week delay and often relatively expensive radiographic imaging. Thus, we need improved, or additional, measures of response, ones that are more closely linked to survival, and it would also help if such measures were both inexpensive and early, *e.g.*, if measures of response could be determined earlier than 6 weeks, Phase II clinical trials could be completed more quickly. In routine practice, an early measure of response could help us recognize ineffective treatment so that we could either change it or simply discontinue it. Such changes might then reduce both the morbidity and costs of treatment.

Ideally, we should base the decision about chemotherapy in advanced non-small cell carcinoma on molecular information gathered from the tumor at the time of diagnosis; however, for the moment, this is not possible. A more primitive yet pragmatic approach is to search for perturbations in the serum proteome after one cycle of treatment. It is relatively easy to sample the serum twice, once before and once after treatment, so that such changes might constitute an early and easily obtained measure of response if they were significantly linked to survival. In the past 10 years, one serum protein marker, CYFRA 21-1 (shortened to CYFRA for the remainder), has shown promise. CYFRA comprises a soluble fragment of cytokeratin 19 with a molecular weight of M_r 30,000, and it has been shown to reflect tumor mass by correlating with tumor stage, survival, and surgical removal (*e.g.*, see Table 1; Refs. 2–27). Recently, Hamzaoui *et al.* (26) published data on CYFRA and response to chemotherapy and found no association between qualitative categories of change in CYFRA and response to chemotherapy. However, a reanalysis of their published data demonstrated that a continuous variable, the ratio logarithm of CYFRA level after the first treatment to the level before treatment, related signifi-

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[2] The abbreviations used are: NSCLC, non-small cell lung cancer; PR, partial remission; HS, hazard score; Ab, antibody.

Table 1 Significant associations between serum level of CYFRA 21-1 and stage, surgical resection, and survival of lung cancer[a]

Author	Year	No.	Stage	Surgery	Survival
Pujol	’93	165	Yes		Yes
Stieber	’93	200	Yes		
Ebert	’94	177	No	Yes	
van der Gaast	’94	212	Yes		
Niklinski	’94	115	Yes		No
Bombardieri	’94	496	Yes		
Molina	’94	136	Yes		
Takada	’95	149	Yes		
Wieskopf	’95	116	Yes		Yes
Moro	’95	105	Yes		Yes
Niklinski	’95	57	Yes		Yes
Niklinski	’95	76	Yes		Yes
Muraki	’96	114	Yes		
Lai	’96	139	Yes		
Ebert	’96	108			Yes
Pujol	’96	314	Yes		Yes
Niklinski	’96	91	Yes		Yes
Szturmowicz	’96	78	No		No
Brechot	’97	116	Yes		Yes
Ebert	’97	129		Yes	
Takei	’97	70	Yes		Yes
Huang	’97	87	Yes	Yes	
Nisman	’98	94	Yes		Yes

[a] Significant increases in stage with increasing levels of CYFRA 21-1 are denoted under the stage column by a “Yes.” Significant drops in CYFRA 21-1 after surgical resection are denoted under the surgery column by a “Yes.” Significant decreases in survival with higher levels of pretreatment CYFRA 21-1 are denoted under the survival column by a “Yes.”

Table 2 Patient characteristics

	No. of patients
Institution	
Washington University	35
Syracuse Upstate Medical University/VAMC	10
Duke University	8
Durham VAMC	5
Gender	
Males: 37	
Females: 21	
Age (years)	
Mean: 65	
Range: 38–83	
Stage	
IIIA: 14	
IIIB: 8	
IV: 36	
Tumor histology	
Adenocarcinoma: 18	
Adenosquamous: 2	
Large cell neuroendocrine: 2	
Large cell: 19	
Squamous: 17	
	No. of patients
Chemotherapy given	
Carboplatin + paclitaxel	29
Carboplatin + gemcitabine	8
Carboplatin + vinorelbine	5
Cisplatin + etoposide	1
Gemcitabine + docetaxel	2
Gemcitabine + irinotecan	1
Vinorelbine + docetaxel	2
Gemcitabine	4
Vinorelbine	4
Paclitaxel	2
Distribution of responses	
PR: 10	
Stable: 29	
Progression: 19	
Status at last follow-up	
Alive with tumor: 23	
Dead of tumor: 35	
Follow-up time (months)	
Median: 8.1	
Range: 1.3–20.6	

cantly to clinical response ($P = 2.08 \times 10^{-7}$). Throughout the remainder, we will symbolize this log ratio as log(y2/y1). The lower the observed log(y2/y1), the higher was the observed likelihood of response. Thus, to test whether the level and log(y2/y1) in CYFRA might provide an early measure of response and relate to survival, we have conducted a pilot study of CYFRA levels on 58 patients with advanced NSCLC and who were treated with chemotherapy. Herein, we report our results.

PATIENTS AND METHODS

Fifty-eight patients with advanced NSCLC untreated previously from four separate institutions affiliated with the Cancer and Leukemia Group B comprise the patients of this study. Seven additional patients were enrolled but excluded from analysis, either because their serum samples were lost or because the second sample was not drawn. All gave their informed consent, and the study was approved by each site’s institutional review board and in accord with an assurance filed with and approved by the Department of Health and Human Services. All were treated with conventional systemic chemotherapy, although the specific choice and number of drugs were left to the discretion of the treating physicians. Thus, our objective was not to test whether CYFRA related to a particular treatment but instead see how it performed as a generic measure of response. All of these patients had measurable or evaluable tumor, and we categorized their response by RECIST criteria (28). All had initial and follow-up computed tomography scans, but mediastinoscopy and positron emission tomography scans were done on just a few patients and when clinically indicated. Eastern Cooperative Oncology Group performance status was 0 for 28 patients, 1 for 18 patients, and 2 for 2 patients, but performance status was not recorded for 10 patients. Other details about the patients, including their stage, tumor histology, treatments, and response, are provided in Table 2.

We obtained two samples of serum: (*a*) the first before the first cycle of chemotherapy; and (*b*) the second immediately before a planned second cycle of chemotherapy. All samples were frozen, stored in freezers at −77 degrees centigrade, and then sent in batches to the central laboratory of Dr. Christenson at the University of Maryland. All were received in good condition. We assayed all samples for CYFRA 21-1 using electrochemiluminescence immunoassay on the ElecSys 2010 system (Roche Diagnostics Corp., Indianapolis, IN). This two-site immunoassay uses two specific monoclonal Ab, KS 19.1 and BM 19.21, to form a [Ab-CYFRA-Ab] sandwich. One Ab is coupled

with a magnetic particle, which is captured in the assay system by the surface of an electrode. The other Ab is labeled with a Ruthenium complex. When electric potential is applied to the electrode, the captured complex emits light, which is detected by a photomultiplier. The assay typically has a variation coefficient of 2–5%.

Statistical Methods. To test whether changes in serum marker after the first treatment related significantly to clinical response, we used the logistic model (29). To relate various variables to survival, we used the Log-rank test for univariate analyses and Cox model for multivariate analyses (30), and because the second serum sample was mandatory, we measured survival time from the time of this sample, *i.e.*, the beginning of the second cycle of chemotherapy. To relate post-treatment levels of CYFRA to pretreatment levels, we used a paired *t* test, and we also used linear regression and ANOVA where indicated. Throughout this study, we relied on the natural logarithm of the raw CYFRA levels, *i.e.*, throughout this study, the term "log" will refer to the natural logarithm. This transformation not only produced a symmetric and nearly normal distribution in the raw data but also in the residuals for the *t* test, linear regression, and ANOVA. Furthermore, by experience, we have found a greater association between the log(CYFRA) and outcomes than between the actual level and outcomes (see "Introduction"). One measure we emphasize here is the difference between the log-(post-treatment CYFRA) and log(pretreatment CYFRA). If we symbolize the level of CYFRA before treatment as y1 and the level of CYFRA after the first cycle of chemotherapy as y2, then this difference can be seen as: log(y2) − log(y1) = log(y2/y1).

Because log(y2/y1) is the logarithm of a ratio, its units no longer relate directly to concentration. All analyses were done with S-PLUS software (2000 version; MathSoft, Inc., Seattle, WA), and all *P*s were for two-sided tests of hypothesis.

RESULTS

Ten patients had PR, 29 had stable disease, and 19 progressed. None experienced a complete response, and 4 did not complete the second cycle of treatment. Other details are included in Table 2. The level of CYFRA before treatment ranged from 0.8 to 1852 ng/ml (mean 37.4), and the level immediately before the second cycle of treatment ranged from 0.8 to 418.5 ng/ml (mean 10.8). The time interval between the two samples averaged 23 days (range: 13–63), and the time interval between first and second treatments averaged 22 days (range: 12–41). The reason these two intervals are not equivalent is because a very few patients, including the 1 with 63 days between the two serum samples, had their first serum sample taken before the first treatment. The initial level of log(CYFRA) related positively and significantly to increasing performance status ($P = 0.0014$ by linear regression), but it did not relate significantly to either clinical stage ($P > 0.1$ by ANOVA) or tumor histology ($P > 0.5$ by ANOVA).

The levels of CYFRA before and after the first cycle of treatment were closely correlated with one another, and this is illustrated in Fig. 1, which shows a logarithm plot of all the pretreatment values of CYFRA on the horizontal axis *versus* the corresponding logarithm of the values after the first cycle on the vertical axis. The line on the plot shows where the points would fall if the two levels of CYFRA were equal, but in fact, most of the points fell below this line, suggesting that for most patients, the first cycle of therapy caused a drop in CYFRA. In fact, the mean value for log(y2/y1) was −0.3117, a result significantly different from 0 ($P = 0.0001$ by paired *t* test), and this difference was not affected by the time interval between samples ($P > 0.1$ by linear regression). The results imply that on average, the value of CYFRA after the first cycle of treatment was 27% less than the value of CYFRA before treatment. Nevertheless, the decrease was not significantly related to clinically measured response ($P > 0.6$ by logistic regression analysis), nor was it related to the treatment used ($P > 0.7$ by ANOVA). For the remainder of this study, we will designate a value of log(y2/y1) < -0.3117 by its equivalent of a drop in serum CYFRA of >27%.

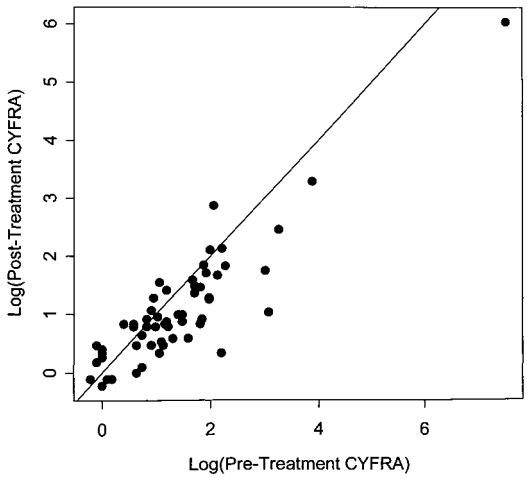

Fig. 1 Plot of natural logarithm of CYFRA before treatment on the horizontal axis *versus* the natural logarithm of CYFRA after the first cycle of treatment on the vertical axis. The *line* shows where the points should fall if the two levels are the same.

Analysis of survival time uncovered more interesting associations. Fig. 2 compares the effects of the CYFRA level and drop on survival to the effects of clinical stage and response. In the figure are four Kaplan-Meier plots. The two left plots deal with the pretreatment variables of clinical stage (top) and level of CYFRA (bottom), and the two right plots deal with the post-treatment variables of clinical PR (top) and log(y2/y1) (bottom). The top left plot shows that stage IV (lowest curve) initially appeared to have a lower survival rate than stages IIIa or IIIb (top two curves), but the differences in survival were not significant by univariate analysis ($P > 0.2$ by Log-rank test). By contrast, the bottom left plot demonstrates more of a separation in estimated survival between those with an initial CYFRA level less than the mean (after log transformation) level of 3.9 ng/ml (top curve) *versus* those with higher levels (bottom curve), and by univariate analysis, this difference was of borderline significance ($P = 0.17$ by Log-rank test). The top right plot shows that initially those with a clinically determined PR (top curve) had improved survival in comparison with those without PR (bottom curve), but by univariate analysis, this difference was not significant ($P = 0.27$ by Log-rank test). The bottom right

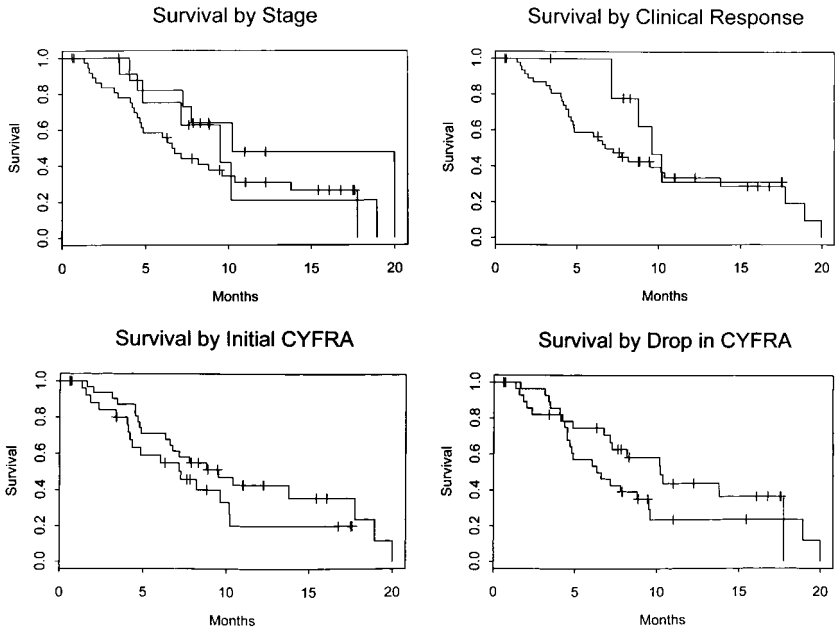

Fig. 2 Four Kaplan-Meier plots of survival probability *versus* time of follow-up in months. *Top left plot,* the survival stratified by clinical stage. Although the top two curves with sparse numbers of patients are for stages IIIa and IIIb, the bottom curve is for stage IV. *Top right plot,* the survival stratified by clinically determined response. Although the top curve is for those with PR, the bottom curve is for those without any response. *Bottom left plot,* the survival stratified by pretreatment level of CYFRA. Although the top curve is for those with CYFRA less than or equal to the mean (after logarithm transformation) value of 3.9 ng/ml, the bottom curve is for those with greater levels. *Bottom right plot,* the survival stratified by the change between pretreatment and post-treatment levels of CYFRA. Although the top curve is for those with a >27% drop in CYFRA such that the magnitude of the log(y2/y1) was less than or equal to the mean of −0.3117, the bottom curve is for those smaller relative decreases.

Table 3 Cox model analysis of overall survival time[a]

	Coefficient	SE	*P*
Stage IIIa			0.14
Stage IIIb			0.16
Response (PR *vs.* none)			0.18
Log(y1)	0.61	0.15	0.000032
>27% Decrease in CYFRA	−1.14	0.41	0.0052

[a] For this analysis, there were 38 uncensored patients. The remaining patients were either lost to follow-up or alive at the time of analysis. Stage IV was used as the default stage, and stages IIIa and IIIb were coded as dummy variables. Log(y1) is the natural logarithm of the pretreatment level of CYFRA. The coefficients and SE are provided for just a two-variable model that included just the last two variables, because the other three were not found to be significant.

plot shows a greater separation between the estimated survival curves for those who experienced a >27% drop in CYFRA (top curve) *versus* those with smaller drops (bottom curve) in CYFRA, but this difference was not by itself significant (P = 0.21 by Log-rank test).

Table 3 shows the results of a Cox multivariable analysis, which examined how clinical stage, clinical response (PR *versus* none), pretreatment level of CYFRA, *i.e.*, log(y1), and change in CYFRA after one cycle of chemotherapy related to overall survival time. Here, log(y1) was used as a continuous variable, and drop in CYFRA was used as a binary variable, *i.e.*, equal to 1 for drops in CYFRA of >27% and otherwise equal to 0. Thus, a value of 1 implied that y2/y1 was <0.73, and a value of 0 implied that y2/y1 was ≥0.73. Stages IIIa and IIIb were coded as dummy variables with stage IV as the default. The results demonstrate that although neither clinical stage nor clinically determined response was significantly related to survival time, both the level of CYFRA and drop in CYFRA related significantly to survival. We also performed a Cox model analysis on the subset of patients with recorded performance status. Once again, both the level of CYFRA and drop in CYFRA related significantly to survival (P = 0.0012 and 0.0053, respectively) after controlling for performance status, which was also significantly related to survival time (P = 0.0085).

In Table 3, the coefficients for the two CYFRA variables are for a two-variable model that excluded the nonsignificant variables of clinical stage and response. These two coefficients allowed us to calculate an HS, to combine the prognostic information provided by log(y1) and drop in CYFRA as follows: HS = 0.61 × log(y1) − 1.14 × r, where r is 1 if the decrease in CYFRA is >27%. Otherwise, it is 0. Fig. 3 shows the impact of HS on estimated survival. When the HS was greater than the mean value of 0.284, the survival was short (median survival was ~5 months from the time of the second CYFRA sample). By contrast, when HS was <0.284, the survival was longer (median survival was ~14 months).

DISCUSSION

Like the studies in Table 1, we have found that the pretreatment level of CYFRA related to subsequent survival time, and our results also suggest that the level of CYFRA in ad-

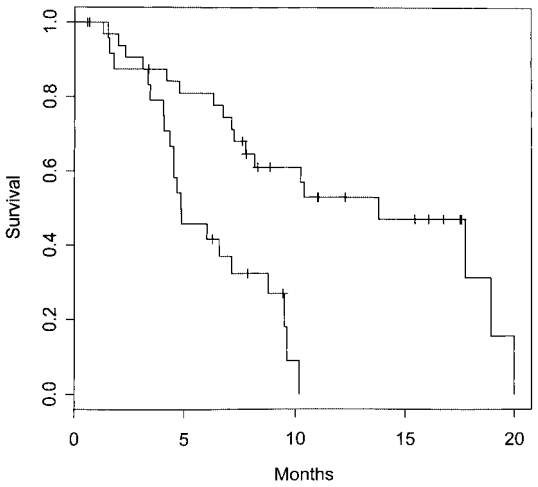

Fig. 3 Kaplan-Meier plots of estimated survival *versus* time in months and stratified by low *versus* high HS, *i.e.*, HS < 0.284 *versus* HS > 0.284.

vanced stage NSCLC may provide more prognostic information than routine clinical stage. Thus, we believe there can be little doubt that the level of CYFRA reflects tumor mass. Furthermore, our results suggest that without controlling for tumor mass, *i.e.*, without controlling for the level of serum CYFRA, one may not discover the importance of a response variable, such as the change in CYFRA with treatment. Specifically, in the Cox model analysis of survival time, the significant relationship between a drop in CYFRA of >27% and survival did not become apparent until the level of CYFRA had been accounted for.

Because the drop in CYFRA was measured after just one cycle of chemotherapy, this change must be an early measure of response. That log(y2/y1) reflects the effect of chemotherapy is clear by its significant drop from 0 to a mean value of -0.3117, implying that for the average patient, chemotherapy decreased the level of CYFRA to ~73% of the pretreatment level. Log(y2/y1) differs from the clinical designation of PR, because it can be determined earlier, does not depend on any radiological exam, and relates more closely to subsequent survival. Log(y2/y1) may also be a more sensitive measure of response, because values <-0.3117 occurred in 48% of our patients, whereas clinical PR occurred in just 17%. Nevertheless, our results suggest that for the drop in CYFRA to impact survival significantly, it must be ≥27%. Should this threshold be validated by additional studies, or be improved on, it could provide an inexpensive early test of the effectiveness of new drugs in NSCLC. Using changes in serum CYFRA as an early response might allow Phase II studies of new chemotherapeutic agents in NSCLC to proceed more quickly and with less toxicity, because studies of ineffective and toxic drugs could close earlier.

Our results suggest that the composite HS using both pretreatment and post-treatment values of CYFRA might be useful as a tool to decide whether or not to continue chemotherapy as opposed to either stopping or changing the therapy. High levels of initial CYFRA coupled with a higher value of log(y2/y1) imply that the HS will be high, and this in turn suggests that the expected survival time after the second assay for CYFRA will be so short that little benefit will derive from continuing the same chemotherapy. If no more effective treatment is available for such patients, then perhaps we should consider just supportive care.

Although we believe that our study is sufficient to justify further testing of CYFRA as a useful tool for treatment decisions in advanced stage NSCLC, we recognize that 58 patients comprise just a pilot study, not a definitive one. Some details remain uncertain, and it is too early to routinely use CYFRA as a sole measure of response in clinical practice. Furthermore, in clinical practice, one would probably want to validate any one assay of a serum marker with a repeat sample. The next step for testing the importance of CYFRA should be a larger study to confirm its importance and increase the certainty about the threshold for a drop in CYFRA, as well as the importance of the HS combining the effects of level and change in CYFRA. Larger numbers of patients may show that both clinical stage and clinically determined response relate significantly to survival, and in that circumstance, one needs to reexamine the contribution made by the initial level of CYFRA, as well as by log(y2/y1). It might be also useful to study the change in CYFRA after additional cycles of treatment. Thus, we plan to assay the level of CYFRA before and after chemotherapy in several clinical trials. If follow-up studies of CYFRA validate the results and optimize the threshold for a drop in CYFRA, then the next step to consider is integrating assays of CYFRA into clinical practice, a step undoubtedly requiring randomized trials. Treatment arms to consider include the continuation of treatment *versus* change in treatment based on, or not based on, changes in the CYFRA, and outcomes to consider include overall survival, frequency of serious morbidities, costs of treatment, and quality of life.

Finally, because CYFRA reflects a small fraction of the serum proteome, we recognize that other soluble serum proteins may provide additional information about response and survival in advanced stage NSCLC. Thus, we favor further research of the serum proteome to identify other proteins that relate to response, and newer technologies such as surface-enhanced laser desorption and ionization time-of-flight mass spectroscopy may help (31).

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