Research Article

iMedPub Journals
www.imedpub.com

Biochemistry & Molecular Biology Journal
ISSN 2471-8084

2020
Vol.6 No.2:5

DOI: 10.36648/2471-8084.6.2.5

Effect of Peripheral Blood Survivin on Survival among Non-Small Cell Lung Cancer Patients: An NCI Experience

Reham A Rashed[1]*, Mohamed Rahouma[2], Mahmoud Morsi[3], Rasha Allam[4], Randa M. Abo Elfetouh[1] and Hala Aziz[5]

1 Department of Clinical Pathology, National Cancer Institute, Cairo University, Egypt
2 Department of Surgical Oncology, National Cancer Institute, Cairo University, Egypt
3 Department of Anesthesia and Surgical ICU, Kasr Alainy School of Medicine, Cairo University, Egypt
4 Department of Biostatistics and Epidemiology, National Cancer Institute, Cairo University, Egypt
5 Department of Medical Oncology, National Cancer Institute, Cairo University, Egypt

***Corresponding author:** Reham A Rashed

Reham_r9@yahoo.com

Department of Clinical Pathology, National Cancer Institute, Cairo University, Egypt.

Tel: 2052619791

Citation: Rashed RA, Rahouma M, Morsi M, Allam R, Randa M, et al. (2020) Effect of Peripheral Blood Survivin on Survival among Non-Small Cell Lung Cancer Patients: An NCI Experience. Biochem Mol Biol Vol.6 No.2:5

Abstract

Background: Survivin is an anti-apoptotic protein and is considered as one of the principle inhibitors of apoptosis (IAP) family which has shown its role in cancer progression, tumor angiogenesis, tumor resistance to chemotherapeutics and radiation. Our goal was to prove the importance of survivin in NSCLC patients and to evaluate if the peripheral blood (PB) survivin level is as prognostic as tissue survivin over expression.

Methods: We enrolled Non-small cell lung cancer patients in the advanced stage prospectively from (2015 to 2017). Real-time PCR was done to detect survivin expression in the blood. Determination of survival time, time to progression of the disease (TTP) and progression free survival (PFS) were the primary outcomes while the other outcomes were to identify the associations between different variables and survivin cutoff, determined by (ROC) curve. Mann Whitney-U and Chi (X^2) were used. To estimate the survival, Kaplan-Meier curves were used and compared using Log-rank. Cox regression was included to identify if survivin was a predictor of survival.

Results: Sixty-six patients with a median age of 55 years (range 47-63.3), 25.8% were females. Adenocarcinoma represented 59.1%. Twelve cases developed progressive disease (PD) among them eight cases had bone metastasis. Median OS, TTP and PFS was 17.1 months (95% CI 13.1-20.9), 11.0 (95% CI 7.3-14.8) and 8.9 (95% CI 8.1-9.8) respectively. The chosen cutoff point for blood survivin level was 3.8 pg/ml (Area under ROC curve=0.644 (95%CI=0.51-0.78), P=0.044) that was associated with better median TTP and PFS of 12.0 vs. 4.9 months and 9.0 vs. 4.9 months in low survivin (≤ 3.8 pg/ml) versus high (>3.8 pg/ml) group (P=0.001 and 0.006) respectively. High Survivin group (>3.8 pg/ml) was associated with worse TTP (Hazard ratio (HR 5.66 (95% CI 1.8-17.7; P=0.003)) and more common to have bone metastasis after PD (100% vs. 26.3% in low survivin (P=0.014).

Conclusion: Survivin is a significant predictor of TTP and PFS in advanced non squamous lung cancer patients. Metastasis is less common in the low survivin group.

Keywords: Lung cancer; Chemotherapy; Survivin; Survival

Received: April 21, 2020; **Accepted:** May 16, 2020; **Published:** May 25, 2020

Introduction

Survivin is one of the principal inhibitors of the apoptosis (IAP) family, it is mainly expressed on tumors and malignant cells with very minimal expression in differentiated adult tissues. It mainly expressed in the G2/M phase of the cell cycle to help rapidly dividing cells while its expression decrease in G1 phase [1]. The mechanism by which the survivin is over expressed or escape the normal gene silencing mechanism is not completely understood but it probably due to alternative splice variants of survivin encoded by BIRC5 gene [2], it has been suggested that survivin inhibit caspases, inhibit P53 protein which a transcription factor

that regulates apoptosis and, inhibits the Smac/Diablo which act as a negative regulator for the XIAP (X-linked inhibitor of apoptosis) [2,3]. Its over expression may enable the cell to bypass the cell cycle checkpoints allowing unregulated cell division that happens in transformed cells [3,4]. This had made its overexpression a possible diagnostic and prognostic marker [5-7]. Significant over expression was found in high grade malignancies as well as late stages of cancer [8-10]. Its over expression in malignant cells was found to be related to more aggressive behavior and worse outcome resulting from chemo-resistance [11,12].

Moreover, survivin may also be involved in tumor angiogenesis besides its direct role in carcinogenesis [13]. Survivin in the tumors cells enhances the B-catenin dependent VEGF transcription, in addition to cellular hypoxia in cancer due to unregulated cellular growth which outgrows the blood supply, cells adapt to hypoxia and stress with the production of hypoxia-induced factors (HIF) and reactive oxygen species which in turn promotes survivin and VEGF expression [14].

Cancer cells have been proved to be found in the patients' blood with early-stage malignant tumors [15]. Thus, searching for these cells in the blood can be of clinical usefulness for early diagnosis, monitoring the therapeutic effect and evaluating the prognosis of a malignant tumor [16].

Studies have exhibited that the detection of survivin expression in the circulating tumor cells (CTCs) in the peripheral blood of lung cancer patients can be significantly correlated to identify early disease stages. Hence, this will help a better follow up being identified as a marker of malignancy, evaluating prognosis, relapse and monitoring therapeutic strategies [17,18].

Being only expressed in most tumors with undetectable amounts in normal tissues, this made survivin inhibition a target in the therapeutic strategy [19]. Several mechanisms have been suggested as inhibition of the survivin protein interactions thus preventing its function, inhibition of the survivin mRNA expression, genre transcription inhibitors and survivin based cancer immunotherapy [20,21].

Thus, our goal here was to evaluate the survivin gene expression in CTCs i.e., the survivin level from peripheral blood of lung cancer patients (NSCLC) and correlate data to its significance in disease progression, response to treatment and survival as well as its potential use as a target molecule in therapy.

Patients and Methods

Sixty-six adult patients with Non-small cell lung cancer in the advanced stage, presented to the oncology clinics of the National Cancer Institute, Cairo University, were prospectively included in this study between April and October 2015 and their follow up for two years after presentation till 2017, together with 30 volunteers as healthy controls. They were classified according to the TNM Classification 7th edition [22]**.** A written consent was given by all patients approved by the national cancer institutes ethical committee [23].

Sample collection and processing

Peripheral blood samples (7 ml) were collected and mononuclear cells were prepared and enriched using density gradient centrifugation, then processed for cell pellet within 1-2 hours of collection, RLT buffer is added to cell pellets and stored in -80°C for the next step of ribonucleic acid (RNA) extraction.

RNA isolation and complementary DNA synthesis

The extraction of total RNA from mononuclear cells was carried out using the QIAamp RNA blood Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. The concentration and purity of RNA were measured at 260 & 280 & 230 nm using Nano Drop 2000/2000c Spectrophotometer (Thermo fisher Scientific, USA). Ratio of A260/A280 = 1.8-2.1 and A260/A230 = 1.8-2.1 indicates highly pure RNA.

Extracted RNA was reverse transcribed into Complementary deoxyribonucleic acid (cDNA) using TaqMan® RNA reverse Transcription Kit (Applied Biosystem, USA) according to the TaqMan RNA Assay protocol. The 20-µl reaction mixture was then incubated at 42°C for 30 min, heated to 95°C for 3 min and the resulting cDNA was stored at -20°C.

Quantitative real-time PCR (RT-PCR) amplification of the survivin gene

In our study, we used the quantitative RT-PCR technique as it showed its accurate reliability and specificity in detecting disseminated tumor cells by assessing a definite cut-off value of the tumor maker transcripts. Furthermore, with continuous measurement of the amplified signals, false positives could be easily detected and discarded increasing the accuracy of the test. Many investigators have reported reliable tumor cell detection by RT-PCR [24].

Amplification of Nucleic acid using (Step One PCR Sequence Detection System supplied by Applied Biosystems AB Co.) The PCR primers and Taqman probes for survivin gene set were developed using Primer Express® software, using the following primers sequences:

Primers: 5'-3' sequences

Survivin –F: AAGAACTGGCCCTTCTTGGA

Survivin –R: CAACCGGACGAATGCTTTT

Survivin Taqman probe: CCAGATGACGACCCCATTGGGCCGG

GAPDH –F: GAAGGTGAAGGTCGGAGTC

GAPDH –R: GAAGATGGTGATGGGATTTC

GAPDH Taqman probe: CAAGCTTCCCGTTCTCAGCC

The PCR reactions were performed and the replicated DNA was detected with dual labeled fluorogenic hybridization probes using the following technique: fluorogenic probes. The reactions were done with the following thermal cycles: 2minutes at 25°C, then 10 minutes followed by 15 seconds both at 95°C and finally 1 minute at 55°C for 45 cycles. For accurate expression of Survivin,

using GAPDH (a house keeping gene) that was used as an internal control for normalization.

The PCR reactions were carried out in a total volume of 25ul for each reaction of which 3ul of cDNA was used 22ul of universal Taqman master mix+ forward, reverse primer and the

Samples in relation to the expression of GAPDH was used to determine the relative expression of both genes (CT method). Failure of the gene expression to reach the software threshold, a sample would be negative and failure of GAPDH amplification, the sample was omitted. The CT is reversely related to the number of target molecules in the reaction.

Data analysis

The auto settings were adjusted to determine the threshold cycle data and baselines. The relative quantification of survivin expression was calculated using the comparative CT method (2–ΔΔCT). The difference of ΔCT value between leukemia and the control is the ΔΔCT (ΔΔCT = ΔCT leukemia gene – ΔCT control gene), and the difference of CT value between the target (gene) and endogenous reference (GAPDH) gene is the ΔCT (ΔCT = CT Target gene – GAPDH gene).

Results

Demographic and clinico-pathological data

66 patients were recruited with a median age of 55 years (IQR: 47- 63.3), 74.2% were males. Adenocarcinoma represented 59.1% while squamous cell carcinoma represented 22.7% and 18.2% others. 56.1% had ECOG-PS ≥2. Platinum containing chemotherapy (CTH) was given in 38 (76.0%) patients, and radiotherapy (RTH) was given in 29 (43.9%) patients. The most common site for RTH was the brain (14/66 (21.2%)) followed by bone (11/66 (16.7%)); **(Table 1)**. Different chemotherapeutic agents were reported in supplementary **(Table 2)**.

The liver was the most common metastatic site in 12/66 (18.2%) **(Table 3).** Twelve cases developed progressive disease (PD) with eight cases had bone metastasis after PD **(Table 4).**

None of the controls showed survivin expression. Median blood

Table 1 Demographics and clinical data comparison among selected cut-off point.

Overall	N (%)	Survivin ≤ 3.8 pg/ml	Survivin >3.8 pg/ml	p-value
Total number	66	60	6	.....
Survivin (median (IQR))	0.45 [0.15, 1.89]	0.38 [0.14, 1.16]	4.39 [4.23, 4.52]	<0.001
Age years (median (IQR))	55.00 [47.00, 63.25]	55.00 [47.00, 63.25]	54.50 [52.50, 55.75]	0.68
Male gender n (%)	49 (74.2)	46 (76.7)	3 (50.0)	0.35
Pathology n (%)				
Adenocarcinoma	39 (59.1)	34 (56.7)	5 (83.3)	0.375
Squamous cell carcinoma	15 (22.7)	14 (23.3)	1 (16.7)	
Others	12 (18.2)	12 (20.0)	0 (0.0)	
Performance status n (%)				
1	25 (37.9)	25 (41.7)	0 (0.0)	0.045
2/3	37 (56.1)	35 (58.3)	6 (100.0)	
Dyspnea n (%)	62 (93.9)	56 (93.3)	6 (100.0)	1
Pain n (%)	47 (71.2)	42 (70.0)	5 (83.3)	0.83
Hemoptysis n (%)	26 (39.4)	20 (33.3)	6 (100.0)	0.006
Cough n (%)	33 (50.0)	30 (50.0)	3 (50.0)	1
Hoarseness of voice n (%)	21 (31.8)	18 (30.0)	3 (50.0)	0.587
Effusion side laterality n (%)				
Absent	29 (43.9)	28 (46.7)	1 (16.7)	0.081
Bilateral	10 (15.2)	10 (16.7)	0 (0.0)	
Unilateral	27 (40.9)	22 (36.7)	5 (83.3)	
Effusion side if unilateral n (%)				
NA	39 (59.1)	38 (63.3)	1 (16.7)	0.002
Left	16 (24.2)	11 (18.3)	5 (83.3)	
Right	11 (16.7)	11 (18.3)	0 (0.0)	
Chest wall mass n (%)	17 (25.8)	17 (28.3)	0 (0.0)	0.306
Presence of metastasis n (%)	47 (71.2)	44 (73.3)	3 (50.0)	0.465
Chemotherapy n (%)	50 (75.8)	44 (73.3)	6 (100.0)	0.34
Platinum containing CTH n (%)	38 (76.0)	35 (79.5)	3 (50.0)	0.28
Progression on Follow Up = PD n (%)	35 (53.0)	31 (51.7)	4 (66.7)	0.785
Response n (%)				
NON-PD	38 (76.0)	33 (75.0)	5 (83.3)	1
PD	12 (24.0)	11 (25.0)	1 (16.7)	
Response n (%)				

Overall	N (%)	Survivin ≤ 3.8 pg/ml	Survivin >3.8 pg/ml	p-value
Non-PD	11 (22.0)	9 (20.5)	2 (33.3)	0.602
SD	16 (32.0)	13 (29.5)	3 (50.0)	
PR	11 (22)	11 (25)	0 (0.0)	
PD	12 (24.0)	11 (25.0)	1 (16.7)	
Death n (%)	24 (36.4)	24 (40.0)	0 (0.0)	0.134
RTH n (%)	29 (43.9)	25 (41.7)	4 (66.7)	0.456
Site of metastasis after PD n (%)				
Others	14 (63.6)	14 (73.3)	0 (0.0)	0.014
Bone	8 (36.4)	5 (26.3)	3 (00.0)	

CTH: Chemotherapy; IQR: Interquartile Range; N: Number; NA: Not-Applicable; NR: Not Reported; PD: Progressive Disease; PR: Partial Response; RD: Regressive Disease; RTH: Radiotherapy; SD: Stable Disease

Table 2 Kaplan Meier survival curves estimated median survival (in months) for survivin sub-groups (≤ 3.8 pg/ml vs. > 3.8 pg/ml): A) Overall Survival (OS), B) Time to Progression (TP) and C) Progression Free Survival (PFS).

Variables	Median survival (months)	Lower CI	Upper CI	P-value
OS (overall)	17.063	13.140	20.986	
- Survivin ≤3.8 pg/ml	17.063			0.156
- Survivin >3.8 pg/ml	NR			
TTP (overall)	11.047	7.327	14.766	------
- Survivin ≤3.8 pg/ml	12.000			0.001
- Survivin >3.8 pg/ml	4.964			
PFS (overall)	8.975	8.149	9.802	--------
- Survivin ≤3.8 pg/ml	9.041			0.006
- Survivin >3.8 pg/ml	4.964			

CI: 95% Confidence Interval, NR: Not Reached, OS: Overall Survival, PFS: Progression Free Survival, TTP: Time to Progression.

Table 3 Predictors of PD (TTP) at different survivin cut-off points.

Variables	P value	HR	95% CI for HR	
			Lower	Upper
Survivin (continuous variable)	.294	1.133	.897	1.432
Survivin >1.00 pg/ml	.709	.878	.443	1.738
Survivin >2.00 pg/ml	.922	.961	.432	2.137
Survivin >2.50 pg/ml	.362	1.445	.655	3.187
Survivin >3.00 pg/ml	.362	1.445	.655	3.187
Survivin >3.50 pg/ml	.591	1.253	.550	2.854
Survivin >3.80 pg/ml	.003	5.657	1.812	17.664
Survivin >4.00 pg/ml	.018	4.576	1.293	16.191

CI: Confidence Interval, HR: Hazard Ratio, PD: Progressive Disease, TTP: Time to Progression.

survivin level was 0.38 (IQR 0.14-1.16) for patients in the low level group versus 4.39 (4.23-4.52) in the high level group. No differences in age or gender, pathology between both cohorts were reported. In high survivin cohort, all cases were ECOG-PS ≥ 2, had hemoptysis and bone metastasis was the main site of metastasis after the progression of disease (P=0.014) **(Table 5).**

Survival and follow-up data

For the median time of follow-up was 13.1 months (95% CI 10.5-15.6). Median OS, TTP and PFS was 17.1 months (95% CI 13.1-20.9), 11.0 (95% CI 7.3-14.8) and 8.9 (95% CI 8.1-9.8) respectively **(Figures 1 and 2).**

Chosen cut-off for blood survivin level was 3.8 pg/ml (Area under ROC curve=0.644 (95%CI=0.51-0.78), P=0.044) that was associated with better median TTP and PFS of 12.0 versus 4.9 months and 9.0 versus 4.9 months in low survivin (≤3.8 pg/ml) versus high (>3.8 pg/ml) groups (P=0.001 and 0.006) respectively. For survivin >3.8 pg/ml, was associated with worst TTP (Hazard rates (HR) 5.66 (95% CI 1.8-17.7; P=0.003). Higher survivin was associated with bone metastasis after PD (100% versus 26.3 in low survivin (P=0.014) **(Table 5).**

Discussion

Survivin is an anti-apoptotic protein that is present and expands uniquely in cancer cells while being almost undetectable in differentiated cells [25-27]. Survivin worsens tumor resistance to various apoptotic stimuli through both the dependent and independent caspase mechanisms [27,28]. On the other side, antagonizing survivin in tumor cells induces apoptosis [28]. As the mode of action of several anti-tumors occurs through

Table 4 Details of chemotherapeutic agents.

Overall	N (%)	Survivin ≤ 3.8 pg/ml	Survivin >3.8 pg/ml	p-value
Platinum containing CTH n (%)	38 (76.0)	35 (79.5)	3 (50.0)	0.28
CTH type n (%)				
• NA	16 (24.2)	16 (26.7)	0 (0.0)	0.125
• CARB-VP16	1 (1.5)	1 (1.7)	0 (0.0)	
• GEM-CIS	11 (16.7)	10 (16.7)	1 (16.7)	
• GEM	1 (1.5)	1 (1.7)	0 (0.0)	
• GEM-CAR	14 (21.2)	14 (23.3)	0 (0.0)	
• NAV	11 (16.7)	8 (13.3)	3 (50.0)	
• NAV-CISP	7 (10.6)	5 (8.3)	2 (33.3)	
• TAX-CARB	5 (7.6)	5 (8.3)	0 (0.0)	
Cycles n (%)				
• NA	16 (24.2)	16 (26.7)	0 (0.0)	<0.001
• 1	5 (7.6)	5 (8.3)	0 (0.0)	
• 2	8 (12.1)	3 (5.0)	5 (83.3)	
• 3	4 (6.1)	4 (6.7)	0 (0.0)	
• 4	3 (4.5)	2 (3.3)	1 (16.7)	
• 6	30 (45.5)	30 (50.0)	0 (0.0)	
2nd Line n (%)				
• NA	30 (45.5)	28 (46.7)	2 (33.3)	0.244
• GEM	3 (4.5)	3 (5.0)	0 (0.0)	
• GEM-CARBO	3 (4.5)	3 (5.0)	0 (0.0)	
• GEM-CIS	2 (3.0)	2 (3.3)	0 (0.0)	
• NAV	4 (6.1)	4 (6.7)	0 (0.0)	
• NO	15 (22.7)	11 (18.3)	4 (66.7)	
• TAX	9 (13.6)	9 (15.0)	0 (0.0)	

CARBO: Carboplatin; CIS: Cisplatin; CTH: Chemotherapy; GEM: Gemcitabine; N: Number; NA: Not-applicable; NAV: Navelbine; TAX: Taxotere

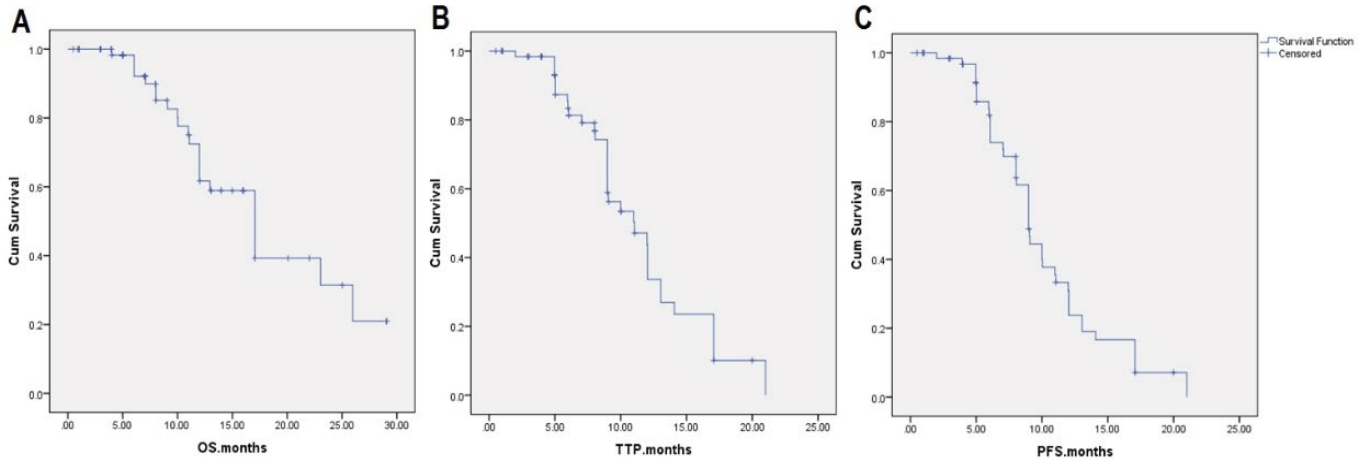

Figure 1 Kaplan Meier survival curves for all cohort (A) Overall Survival (OS), (B) Time to Progression (TP) and (C) Progression Free Survival (PFS).

activating apoptosis, survivin expression may play a role in the resistance of anticancer agents helping to predict response to chemotherapy [26].

Most of the studies focused on survivin expression in the tumor at diagnosis, the positive tissue expression of survivin correlates with more aggressive behavior and poorer prognosis of the tumor [25,29-31]. For this study, we sought to investigate the criteria of low survivin levels in peripheral blood of patients at presentation and compare them to the high group to evaluate if the role of blood survivin is as prognostic as tissue survivin overexpression. We studied 66 cases presenting with NSCLC to the national cancer institute over the period from April to October 2015 and was followed up for 2 years. The median age of cases was 55 years. Regarding the histological types, Adenocarcinoma represented 59.1% of cases. While regarding TNM staging 71% are stage IV, there is conflicting evidence regarding the correlation of survivin

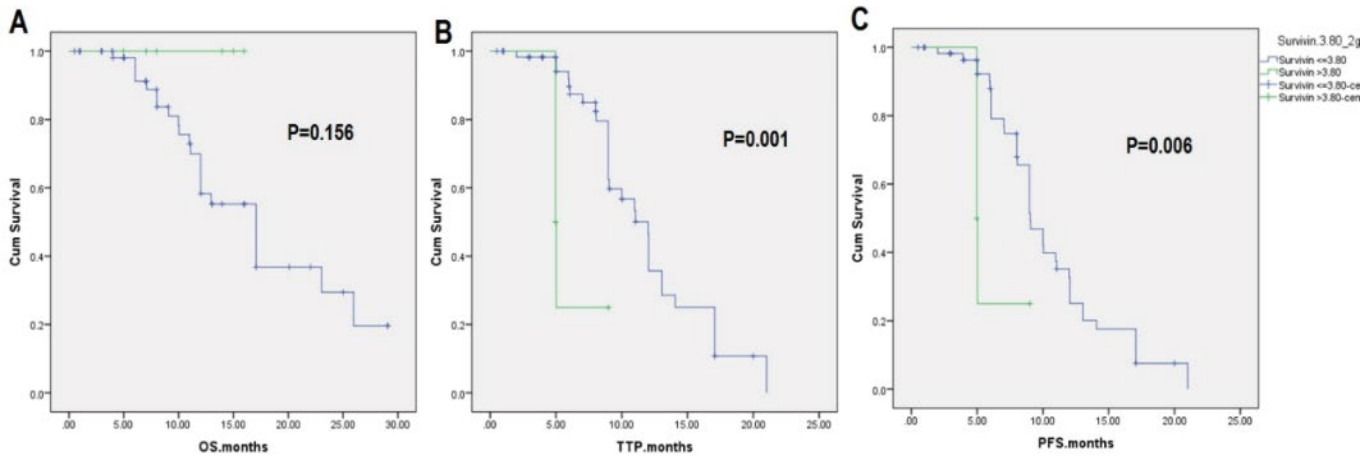

Figure 2 Kaplan Meier survival curves for surviving subgroups (≤ 3.8 pg/ml vs > 3.8 pg/ml): (A) Overall Survival (OS), (B) Time to Progression (TP) and (C) Progression Free Survival (PFS).

Table 5 Site of metastasis at the start of treatment and after PD based on survivin cut-off.

Variables	N (%)	Survivin ≤ 3.8 pg/ml	Survivin >3.8 pg/ml	p-value
Metastatic site n (%)				
• NR	19 (28.8)	16 (26.7)	3 (50.0)	0.17
• Oseous/Finger	1 (1.5)	1 (1.7)	0 (0.0)	
• Adrenal	1 (1.5)	1 (1.7)	0 (0.0)	
• Ascites+Pulmonary	1 (1.5)	1 (1.7)	0 (0.0)	
• Bone	3 (4.5)	1 (1.7)	2 (33.3)	
• Brain	5 (7.6)	4 (6.7)	1 (16.7)	
• CX LND	10 (15.2)	10 (16.7)	0 (0.0)	
• Liver	12 (18.2)	12 (20.0)	0 (0.0)	
• Liver/Bone	2 (3.0)	2 (3.3)	0 (0.0)	
• Liver/SCLAV	1 (1.5)	1 (1.7)	0 (0.0)	
• Lung	1 (1.5)	1 (1.7)	0 (0.0)	
• PERIC EFF/SC NOD	2 (3.0)	2 (3.3)	0 (0.0)	
• Pleural	3 (4.5)	3 (5.0)	0 (0.0)	
• SC NOD	5 (7.6)	5 (8.3)	0 (0.0)	
Site of metastasis after PD n (%)				
• Other metastasis	14 (73.7)	14 (73.3)	0 (0.0)	0.036
• Bone metastasis	5 (26.3)	5 (26.3)	3 (100.0)	

CX: Cervical; LND: Lymph Node; N: Number; NOD: Node; NR: Not Reported; PD: Progressive Disease; PERIC EFF: Pericardial Effusion; SC: Subcutaneous; SCLAV: Supraclavicular

expression with the histological type of cancer and TNM stage [25]. In our cases, the lung cancer incidence rates in females (26%) were lower than in males which come in concordance to previously published by Li et al. [10].

We choose the cut off 3.8 pg/ml because at this point the median TTP in the low group (survivin <3.8 pg/ml) vs. high group (survivin >3.8 pg/ml) were (12 vs. 4.96 months, P =0.001) respectively, while median PFS in low vs. high groups were (9 vs. 4.96 months, P =0.006) respectively. The low survivin levels (below the cut off) was present in 60 cases. In Naumnik et al. study, the cut off value for survivn was 81.92 pg/ml, he showed no difference in the probability of survival between high and low groups. In a study, Serum cutoff value for ovarian cancer was 29.8 pg/ml, No et al. showed a significant disease free survival for the low group than high group over a follow up period of 20 months [32]. In Dong study, the cutoff value was set 72.58 pg/ml, where low

levels were present in (36.3%) and high levels in (63.7%) [33]. Although Dong didn't report PFS or TTP, he found that there was a significant overall survival difference associated with the level of serum survivin levels which we didn't find in case of the OS. A study done by Ganuldi et al. where he showed that serum survivin level was determined at ≥120.8 pg/ml where the risk ratio for survivin ≥120.8 pg/ml to predict cancer was 4.198, he also documented that serum survivin levels were not influenced by gender, size, type of cancer, metastasis status, liver metastasis status, mortality, presence or absence of leukocytosis, anemia, thrombocytosis, and high tumor markers, but an important thing to be mentioned is that Ganuldi didn't include patients with lung cancer [34]. However, Unlike Ganuldi, No et al. mentioned a positive relationship between survivin levels with age [32]. Serum survivin Cutoff values where 13.7 pg/ml in Hepatocellular carcinoma (HCC), 110 pg/ml in ovarian cancers; 15.18 pg/ml in ALL, but none of these studies compared high and low serum survivin levels or reported a follow-up period [35-37]. Guney et al. did a study where he showed no significant difference in serum and urine survivin levels in patients with breast cancer [38].

As regard survival analysis, our median overall survival was (17 months) in the group with low survivin with comparison to Dong et al. [33] who studied pancreatic cancer where the overall survival was (26 months) in the low level group while it was 9 month in the high group.

Regarding the relation between the different survivin levels and metastasis, our study, higher serum survivin levels are associated with a high incidence of bony metastasis. Similar to our study, No et al. in his study about Ovarian cancer showed high survivn levels with positive peritoneal cytology and omental metastasis of ovarian cancer [32]. Dong et al. showed in his study where serum survivin levels were higher in patients with perineural invasion, poor lymph node status, and venous invasion, compared with those without [33]. Also, Guney showed significantly higher survivin levels in patients with nodal invovlvment but not metastasis [38]. Most of the studies which reported the relation of survivin expression to metastasis as zhu et al. ye et al. and Cui et al. where all focusing on tissue survivin expression and not serum levels [39-41].

Goricar et al. reported that patients with progressive cancer have a higher survivin level at the time of diagnosis but even higher levels before treatment have no effect on PFS and OS. In contrast, if the level of survivin increased after radiotherapy or chemotherapy, indicating a better effect of chemotherapy, better OS and PFS making survivin a good indicator for the response to treatment. The same data was reported by 2 other studies [42,43].

When we evaluated survivin levels in our patients, the response rate was comparable in both low and high groups at presentation to platinum based CTH. The same result was reported by Numanik et al. but unlike our study, he also reported that the blood level of survivin in NSCLC patients was comparable to controls and there is no correlation between its level and histological type or stage of lung cancer [44]. The same also was reported by Goksel et al. [45]. However, studies done with Kapellos et al. and Yie et al. [46] showed an increased survivin expression in the blood of patients with aggressive NSCLC. Our study with the given survivin expressions, confirms that high expression indicates more aggressive cancer and poor prognosis.

Finally, although this is an analysis of advanced NSCLC among patients attending tertiary referral center, this study showed some limitations; low studied number and absence of data about Epidermal Growth Factor Receptor (EGFR) mutations, EML4-ALK, Excision repair cross-complementing 1 (ERCC1), or ribonucleotide reductase M1 (RRM1) in this study that had been reported to affect outcomes in advanced NSCLC [47,48].

Conclusion

Our study suggests that peripheral blood survivin level is an important marker for the prognosis of NSCLC by RT-PCR technique. Further Prospective studies are needed with a larger number of cases to define the possible prognostic role of survivin. Survivin is a significant predictor of TTP and PFS in advanced cases of non-squamous lung cancer patients. Metastasis is less common in the low survivin group. Good response to chemotherapy, better OS and PFS were reported in advanced NSCLC cases even no statistical significance.

Ethics Approval and Consent to Participate

Study was approved by the ethical committee.

Consent for Publication

Yes.

Competing Interests

The authors declare that they have no competing interests.

Availability of Data and Materials

The datasets analyzed during the current study is available from the corresponding author on reasonable request.

Authors' Contributions

Concept: RAR, MR, HA; Writing: All authors; Statistical analysis: MR; Revision and edits: All authors.

References

1 Jaiswal PK, Goel A, Mittal RD (2015) Survivin: A molecular biomarker in cancer. Indian J Med Res 141:389–397.

2 Chen X, Duan N, Zhang C, Zhang W (2016) Survivin and Tumorigenesis: Molecular Mechanisms and Therapeutic Strategies. J Cancer 7: 314–323.

3 Sah NK, Khan Z, Khan GJ, Bisen PS (2006) Structural, functional and therapeutic biology of survivin. Cancer Letters 244: 164–171.

4 Garg H, Suri P, Gupta JC, Talwar GP, Dubey S (2016) Survivin: a unique target for tumor therapy. Cancer Cell International 16: 49.

5 Skarin AT, Herbst RS, Leong TL, Bailey A, Sugarbaker D (2001) Lung cancer in patients under age 40. Lung Cancer 32: 255–264.

6 Mauri D, Pentheroudakis G, Bafaloukos D, Pectasides D, Samantas E, et al. (2006) Non-small cell lung cancer in the young: a retrospective analysis of diagnosis, management and outcome data. Anticancer Res 26: 3175–3181.

7 Radzikowska E, Roszkowski K, Głaz P (2001) Lung cancer in patients under 50 years old. Lung Cancer 33: 203–211.

8 Nishino M, Jackman DM, Hatabu H, Yeap BY, Cioffredi LA, et al. (2010) New Response Evaluation Criteria in Solid Tumors (RECIST) guidelines for advanced non-small cell lung cancer: comparison with original RECIST and impact on assessment of tumor response to targeted therapy. AJR Am J Roentgenol 195: 221-228.

9 Edge SB, Compton CC (2010) The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 17: 1471–1474.

10 Li J, Wei Z, Li H, Dang Q, Zhang Z, et al. (2015) Clinicopathological significance of fibroblast growth factor 1 in non-small cell lung cancer. Hum Pathol 46: 1821–1828.

11 Dempke WC (2015) Targeted Therapy for NSCLC-A Double-edged Sword? Anticancer Res 35: 2503–2512.

12 Smit EF, Van Meerbeeck JP, Lianes P, Debruyne C, Legrand C, et al. (2003) Three-arm randomized study of two cisplatin-based regimens and paclitaxel plus gemcitabine in advanced non-small-cell lung cancer: A phase III trial of the European Organization for Research and Treatment of Cancer Lung Cancer Group--EORTC 08975. J Clin Oncol 21: 3909–3917.

13 Mark JB (2001) General thoracic surgery, Fifth Edition: Edited by Thomas W. Shields, MD, Joseph LoCicero III, MD, and Ronald B. Ponn, MD, Philadelphia, Lippincott Williams & Wilkins. The Annals of Thoracic Surgery 71: 380.

14 Sanhueza C, Wehinger S, Castillo Bennett J, Valenzuela M, Owen GI, et al. (2011) The twisted survivin connection to angiogenesis. Molecular Cancer 14: 198.

15 Mamdani H, Ahmed S, Armstrong S, Mok T, Jalal SI (2017) Blood-based tumor biomarkers in lung cancer for detection and treatment. Transl Lung Cancer Res 6: 648–660.

16 Alix-Panabières C, Riethdorf S, Pantel K (2008) Circulating tumor cells and bone marrow micrometastasis. Clin Cancer Res 14: 5013–5021.

17 Falleni M, Pellegrini C, Marchetti A, Oprandi B, Buttitta F, et al. (2003) Survivin gene expression in early-stage non-small cell lung cancer. J Pathol 200: 620–626.

18 Kren L, Brazdil J, Hermanova M, Goncharuk VN, Kallakury BV, et al. (2004) Prognostic significance of anti-apoptosis proteins survivin and bcl-2 in non-small cell lung carcinomas: a clinicopathologic study of 102 cases. Appl Immunohistochem Mol Morphol 12: 44–49.

19 Singhal S, Vachani A, Antin-Ozerkis D, Kaiser LR, Albelda SM (2005) Prognostic implications of cell cycle, apoptosis, and angiogenesis biomarkers in non-small cell lung cancer: a review. Clin Cancer Res 11: 3974–3986.

20 Li F, Aljahdali I, Ling X (2019) Cancer therapeutics using survivin BIRC5 as a target: what can we do after over two decades of study? J Exp Clin Cancer Res 38.

21 Groner B, Weiss A (2014) Targeting Survivin in Cancer: Novel Drug Development Approaches. Bio Drugs 28: 27–39.

22 Mirsadraee S, Oswal D, Alizadeh Y, Caulo A, Van Beek EJ (2012) The 7th lung cancer TNM classification and staging system: Review of the changes and implications. World J Radiol 4: 128–134.

23 WMA (2019) The World Medical Association-WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects.

24 Zieglschmid V, Hollmann C, Böcher O (2005) Detection of disseminated tumor cells in peripheral blood. Crit Rev Clin Lab Sci 42: 155–196.

25 Kapellos G, Polonifi K, Farmakis D, Spartalis E, Tomos P, et al. (2013) Overexpression of survivin levels in circulation and tissue samples of lung cancer patients. Anticancer Res 33: 3475–3480.

26 Goričar K, Kovač V, Franko A, Dodič-Fikfak M, Dolžan V (2015) Serum Survivin Levels and Outcome of Chemotherapy in Patients with Malignant Mesothelioma [Internet]. Disease Markers.

27 Li D, Hu C, Li H (2018) Survivin as a novel target protein for reducing the proliferation of cancer cells. Biomed Rep 8: 399–406.

28 Fukuda S, Pelus LM (2006) Survivin, a cancer target with an emerging role in normal adult tissues. Mol Cancer Ther 5:1087–1098.

29 Ryan BM, Konecny GE, Kahlert S, Wang HJ, Untch M, et al. (2006) Survivin expression in breast cancer predicts clinical outcome and is associated with HER2, VEGF, urokinase plasminogen activator and PAI-1. Ann Oncol 17: 597–604.

30 Als AB, Dyrskjøt L, von der Maase H, Koed K, Mansilla F, et al. (2007) Emmprin and survivin predict response and survival following cisplatin-containing chemotherapy in patients with advanced bladder cancer. Clin Cancer Res 13: 4407–4414.

31 Sarela A, Macadam R, Farmery S, Markham A, Guillou P. (2000) Expression of the antiapoptosis gene, Survivin, predicts death from recurrent colorectal carcinoma. Gut 46: 645–650.

32 No JH, Jeon YT, Kim YB, Song YS (2011) Quantitative detection of serum survivin and its relationship with prognostic factors in ovarian cancer. Gynecol Obstet Invest 71: 136–140.

33 Dong H, Qian D, Wang Y, Meng L, Chen D, et al. (2015) Survivin expression and serum levels in pancreatic cancer. World J Surg Oncol 13: 189.

34 Gunaldi P (2020) The value of serum survivin level in early diagnosis of cancer.

35 El-Attar HA, Kandil MH, El-Kerm YM, El-Ghandour MK (2010) Comparison of serum survivin and alpha fetoprotein in Egyptian patients with hepatocellular carcinoma associated with hepatitis C viral infection. Asian Pac J Cancer Prev 11: 897–903.

36 Dobrzycka B, Mackowiak-Matejczyk B, Terlikowska KM, Kulesza-Bronczyk B, Kinalski M, et al. (2015) Prognostic significance of

pretreatment VEGF, survivin, and Smac/DIABLO serum levels in patients with serous ovarian carcinoma. Tumour Biol 36: 4157–4165.

37 Ahmed MB, Shehata HH, Moussa M, Ibrahim TM (2012) Prognostic significance of survivin and tumor necrosis factor-alpha in adult acute lymphoblastic leukemia. Clin Biochem 45: 112–116.

38 Guney N, Soydine HO, Derin D, Camlica H, Duranyildiz D, et al. (2006) Serum and Urine Survivin Levels in Breast Cancer. MO 23: 335–340.

39 Zhu H, Chen XP, Zhang WG, Luo SF, Zhang BX (2005) Expression and significance of new inhibitor of apoptosis protein survivin in hepatocellular carcinoma. World J Gastroenterol 11: 3855–3859.

40 Cui F, Chen B, Chen J, Huang Y, Luo R (2008) Expressions of Survivin and vascular endothelial growth factor in hepatocellular carcinoma and their clinical significance. 28: 761–763.

41 Ye CP, Qiu CZ, Huang ZX, Su QC, Zhuang W, et al. (2007) Relationship between survivin expression and recurrence, and prognosis in hepatocellular carcinoma. World J Gastroenterol. 13: 6264–6268.

42 Farnebo L, Tiefenböck K, Ansell A, Thunell LK, Garvin S, et al. (2013) Strong expression of survivin is associated with positive response to radiotherapy and improved overall survival in head and neck squamous cell carcinoma patients. Int J Cancer 133: 1994–2003.

43 Freier K, Pungs S, Sticht C, Flechtenmacher C, Lichter P, et al. (2007) High survivin expression is associated with favorable outcome in advanced primary oral squamous cell carcinoma after radiation therapy. Int J Cancer 120: 942–946.

44 Naumnik W, Nilklińska W, Ossolińska M, Chyczewska E (2009) Serum levels of HMGB1, survivin, and VEGF in patients with advanced non-small cell lung cancer during chemotherapy. Folia Histochem Cytobiol 47: 703–709.

45 Goksel G, Taneli F, Uslu R, Ulman C, Dinc G, et al. (2007) Serum her-2/neu and survivin levels and their relationship to histological parameters in early-stage breast cancer. J Int Med Res 35: 165–172.

46 Yie S, Lou B, Ye S, He X, Cao M, et al. (2009) Clinical significance of detecting survivin-expressing circulating cancer cells in patients with non-small cell lung cancer. Lung Cancer 63: 284–290.

47 Schveigert D, Cicenas S, Bublevic J, Askinis R, Sapoka V, et al. (2014) The role of genetic and other biomarkers in NSCLC prognosis. Cent Eur J Med 9: 382–390.

48 Rahouma M, Yehia M, Abdelrahman A (2010) P2.03a-034 RRM1 - A Prognostic Marker in Advanced NSCLC among Male Smokers Receiving Chemotherapy. Journal of Thoracic Oncology 12: 908–909.