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Peripheral Blood Platelet Counts Identify Prognostically Diverse Clinical Phenotypes in Hepatocellular Carcinoma

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Keywords: HCC; Size; Biomarkers; Survival.

Abstract

Background: The factors responsible for Hepatocellular Carcinoma (HCC) growth are not precisely known.

Aims: To study the clinical parameters associated with increases in Maximum Tumor Diameter (MTD).

Methods: A new cohort of 944 prospectively accrued HCC patients was analyzed for large size associations.

Results: Patients were ordered into MTD terciles. Blood platelets, GGT and AST levels significantly increased and total bilirubin decreased with increase in MTD. Similar results were found only for platelets, in patients with low Alpha-Fetoprotein (AFP) levels, for whom biomarkers are scanty. Survival significantly decreased for patients with high platelet or GGT levels, even when AFP levels were low.

Comparison of patients with low and high platelet levels showed that in the <6cm MTD group, patients with higher platelet numbers had lower total bilirubin and AST, and higher albumin, hemoglobin and percent patients with Portal Vein Thrombosis (PVT) than those with lower platelets. Univariable logistic analysis on HCCs >6cm versus <6cm revealed significantly higher odds ratios for elevated blood platelet, AFP, GGT and ALKP levels. Cox regression analysis on death showed that in <6cm MTD patients, significant hazard ratios were for platelets, GGT, AFP, ALKP and PVT; but not for >6cm MTD patients, suggesting different mechanisms. Given the association of higher platelets with larger tumors and good liver function, their precursors are suggested to be small tumors with higher platelets and endogenous tumor factors. However, patients with low platelets and larger HCCs might have a different HCC lineage, likely associated with liver inflammation factors.

Conclusions: Blood platelet levels are a potential marker for HCC phenotype and prognosis, including in patients with low AFP. They may also be a therapeutic target.

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Introduction

Hepatocellular Carcinoma (HCC) is thought to arise in large part in a liver that has been chronically inflamed for many years or decades, most typically as a result of long-term infection by hepatitis B or C virus, alcoholism or obesity-associated chronic liver disease [1]. The chronic inflammation often causes fibrosis with resulting cirrhosis and portal hypertension. The latter has several clinical sequelae, that include thrombocytopenia, which has been suggested to be a cirrhosis surrogate [2]. Thus, when HCC eventually develops, many of the patients have 2 diseases, namely cirrhosis and HCC, either or both of which independently or together can lead to patient death [3], with cirrhosis causing death by inflammation-associated liver failure and HCC causing death by cancer (A tale of 2 diseases: ref 4), perhaps through oncogenes or other growth factors [5,6]. Some HCC studies have shown that blood platelet levels can discriminate between cirrhosis-associated and not-associated HCC, with resultant differences in clinical characteristics at HCC diagnosis as well as in clinical outcomes [7-9]. Capitalizing on evolving knowledge relating to the pathogenic role of platelets in hepatocarcinogenesis, this study intends to investigate whether platelet counts might be a non invasive biomarker capable of defining phenotypically different disease entities in HCC, with a particular emphasis on the relation to Maximum Tumor Diameter (MTD) and macroscopic portal vein thrombosis (PVT), as well as on patient survival. leading to the suggestion that platelet-defined portal hypertension helps identify HCC phenotypes.

Materials and Methods

Clinical characteristics of the study population

A database based on the Inonu university weekly liver tumor board was prospectively created for baseline tumor characteristics, which included 944 adult patients with HCC and were assigned to potential transplant, other ablative interventions, or to non-surgical management. Baseline radiological tumor characteristics of Maximum Tumor Diameter (MTD), number of tumor nodules, presence of macroscopic tumor Portal Vein Tumor Thrombosis (PVT) were assessed from CAT scans taking into account arterially enhancing sequences as per AASLD/EASL diagnostic guidelines. Baseline lab parameters included serum Alpha-Fetoprotein (AFP) levels, standard liver function tests and routine clinical hematology parameters. The diagnosis of HCC followed histological or imaging criteria according to AASLD/EASL guidelines.

Surgical patients were treated mainly by liver transplantation (with a small number by resection, n=4), while the majority of patients received systemic therapy (medical oncology referrals for Sorafenib), locoregional therapy (chemoembolization or radioembolization in interventional radiology), and a very few could only receive best supportive care (n=7).

Statistical Analysis

The normality of the quantitative data was assessed by the Shapiro-Wilk and Kolmogorv-Smirnov tests. Quantitative data were summarized by median, minimum, and maximum values. Mann-Whitney U test was applied for the comparison of two independent groups. For more than two group comparisons, the Kruskal-Wallis test and the Conover pairwise comparison method were used. Qualitative data were expressed by count and percentage, and comparisons were performed by Pearson's chi-square test or continuity-corrected chi-square test where appropriate. Univariable and multivariable logistic regression analyses were used for Odds Ratio (OR) estimations. Survival comparisons were performed by the Kaplan-Meier method and the Log-rank test. Hazard Ratio (HR) estimations were achieved by univariable and multivariable Cox regression analyses. In all analyses, two-sided significance level was considered to be 0.05.

Ethical approval

Database management conformed to legislation on privacy, and this study conforms to the ethical guidelines of the Declaration of Helsinki and approval for this retrospective study on deceased cases and de-identified patients with HCC. This work was approved by our Institutional Ethics Committee (Institutional Review Board Approval No. 2022-3905) for a waiver from obtaining written informed consent for de-identified and mostly deceased patients, in accordance with local guidelines.

Results

Parameter levels in relation to MTD terciles

The Maximum Tumor Diameter (MTD) tercile approach has been useful in the study of platelets in relation to MTD in another HCC cohort [10,11], for which survival data was not available. In the current study, we have expanded on the previous one, by incorporating additional blood parameters as well as survival (Figures 1 and 2). We found that blood platelet levels significantly increased in patients with the highest MTD tercile (Figure 1A1). GGT levels also showed an increase in the highest MTD tercile (Figure 1B). AST levels showed an increase with MTD (Figure 1C), and total bilirubin levels were significantly higher for the lowest MTD tercile and lowest for the highest MTD tercile (Figure 1D). Albumin levels increased with each tercile, but not significantly (Figure 1E) and AFP levels increased with increase in MTD, as expected, but also not significantly (Figure 1F).

An identical approach was taken for the cohort of patients with low serum AFP, as they constitute approximately half of all HCCs in other series [12,13]. Analysis by platelet levels showed a significant increase as MTD increased (Figure 1A2). GGT levels also increased with each increase in MTD tercile, though not significantly. These platelet findings may be useful, considering the limited availability of biomarkers in low AFP HCC patients. The results showed no clear association with MTD tercile for the other parameters in low AFP patients, namely AST, AFP, total bilirubin and albumin (data not shown).

Parameter levels in relation to survival

The relationship of parameter levels to both cumulative and median survival was next examined (Figure 2). There was a significant relationship between platelets and survival (Figure 2A), with a 3-fold survival range between lowest blood platelet levels (longest survival) and highest levels, (shortest survival), log-rank p-value <0.001. A similar relationship and significance was found for blood GGT (Figure 2bB) and AFP levels (Figure 2C), p-value for each parameter <0.001. However, there was no relationship between blood albumin levels and survival, and although there was a relationship between ALKP levels and survival, it was not significant (data not shown). The relationship between platelets and survival was then re-examined for patients with low AFP levels, which was also significant (Figure 2D), log-rank p-value <0.001. There was a 4-fold survival range between patients with lowest and highest platelet values. Similar results were found for GGT levels in low AFP patients (Figure 2E), p-value 0.003. These survival associations for both platelet and GGT levels in patients with low AFP levels, maybe be useful

for clinical practice.

Clinical characteristics of patients with high or low blood platelet counts, stratified by MTD.

Since only platelets were significantly related both to MTD and to survival in Figures 1 and 2 in presence and absence of low AFP levels, their relationships to other clinical parameters were examined further. In order to try and understand the contribution of platelet counts to MTD, the clinical characteristics of patients were compared in the 2 dichotomized platelet groups (Table 1). Examination of patients in the low and high platelet subsets in the ≤6cm MTD group (Table 1 top half) showed significant differences in platelet levels (by definition), with the low platelet group having significantly higher total bilirubin, AST and ALKP, lower albumin, hemoglobin, neutrophil and lymphocyte levels, all consistent with cirrhotic portal hypertension, compared to the high platelet subset (Table 1, top half). With respect to tumor characteristics, the low platelet group had a significantly lower MTD and significantly lower percent of patients with macroscopic Portal Vein Thrombosis (PVT) than the high platelet group. AFP levels were lower for the low platelet group, but not significantly. Survival was not significantly different between the 2 platelet subgroups, either for transplant surgery or no-surgery. Unsurprisingly, the survival of the no-surgery patients was much lower than for the surgery groups, in both platelet groups.

For the MTD >6cm patients (Table 1, lower half), the trends in liver function were similar to the ≤ 6cm MTD group (more portal hypertension in the low platelet group). The percent of patients with PVT and the median MTDs were each higher in the high platelet group compared to the low platelet group, but not significantly. Survival was not significantly different between the 2 platelet groups.

Interestingly, the ratio of patients with low to high platelet counts in the ≤6cm MTD group was 387:394 (or almost 1:1); and the patient ratio in the >6cm MTD group was 59:104 (or almost 1:2). There was thus a shift in the ratios, with a greater percent of patients having high platelet levels for the larger MTD group.

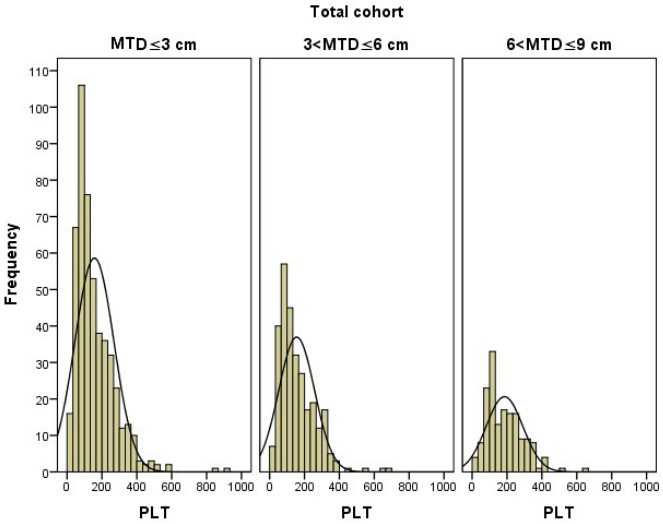
Logistic regression analysis for factors associated with MTD>6cm.

A logistic regression analysis was calculated using the forward selection method, for Odds Ratios (OR) for patients having an MTD >6cm versus the reference MTD of ≤6cm (Table 2A). Significant factors with the highest ORs on univariable regression were platelets >125,000 (OR 2.145), AFP levels >20 IU/mL (OR 1.328), GGT levels >60 IU/mL (OR 1.411) and ALKP levels >120 IU/mL (OR 1.344). Platelets and AFP were also significant on multivariable analysis. Albumin with OR 1.273 was also significant on univariable analysis. A model was then constructed for the 4 parameters together that had the highest ORs (Table 2B). Patients with high platelets plus high AFP plus high GGT plus high ALKP had an OR of 4.014 when compared to the combination of the same parameters having with low values (reference), p<0.001.

Survival analysis in patients with small and large size HCCs

A Cox regression analysis on death was then constructed, separately for HCCs with MTD ≤6cm (Table 3A) and with MTD >6cm (Table 3B). For HCCs with MTD ≤6cm, 4 parameters had a Hazard Ratio (HR) of >1.5. They were: platelets (HR 1.613), AFP (HR 1.530), GGT (HR 1.753) and PVT (HR 1.769). For HCCs with MTD >6cm, only 2 parameters had an HR of >1.5. They were presence of HBV (HR 1.784) and ALKP (HR 1.742), with ALKP also being significant in the multinomial analysis. Low albumin had a protective effect (HR 0.716), although not quite significant. Neither PVT nor AFP was significant for patients with the larger HCCs (unlike for patients with smaller tumors). Thus, different parameters were significant for survival in the patients with small and large HCCs. For small tumors, they included liver factors (platelets, GGT) and tumor factors (AFP, PVT), while for large tumors, only HBV and ALKP (with a protective effect for albumin). These suggest the possibility of different mechanisms involved for small and large HCC survival. Why AFP and PVT are not so important prognostic factors for patients with large HCCs is unclear. Perhaps large HCC mass over-rides the importance of other factors. But that only suggests other questions.

The 3 parameters with high HR for the small tumors were then combined, separately in patients who had PVT or its absence (Table 3C, top 3 rows). Patients with high levels of the combination of platelets, AFP and GGT had significantly higher HRs than patients with low values of these 3 parameters. This was found both for patients with or without PVT. For patients with large tumors, the combination of low ALKP and high (normal) albumin levels was compared to the opposite of high ALKP and low albumin levels. Patients with high ALKP plus low albumin levels had a significantly higher HR (2.5) than the reference group in the total cohort and the PVT- cohort, although insufficient patient numbers precluded separate examination of PVT+ patients.

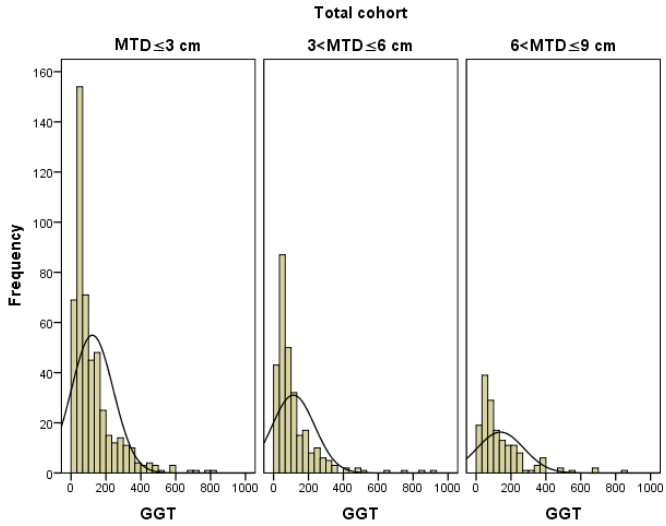


MTD tercile analysis for platelet levels in patients in the total cohort.

	MTD≤3 cm		3<MTD≤6 cm		6<MTD≤9 cm		
A1.	n	Median (min.max.)	n	Median (min.max.)	n	Median (min.max.)	p
PLT	496	112 (11-920)[a]	285	125 (2.64-691)[a]	163	168 (26-653)[b]	<0.001

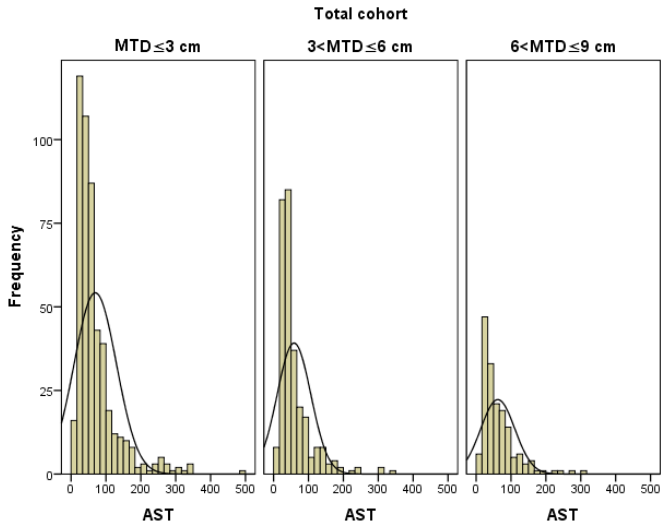
MTD tercile analysis for platelet levels in patients with low blood AFP (<200 IU/mL) levels

	MTD≤3 cm		3<MTD≤6 cm		6<MTD≤9 cm		
A2.	n	Median (min.max.)	n	Median (min.max.)	n	Median (min.max.)	p
PLT	350	109 (11.2-920)[a]	192	115 (2.64-640)[a]	102	160 (31-653)[b]	<0.001



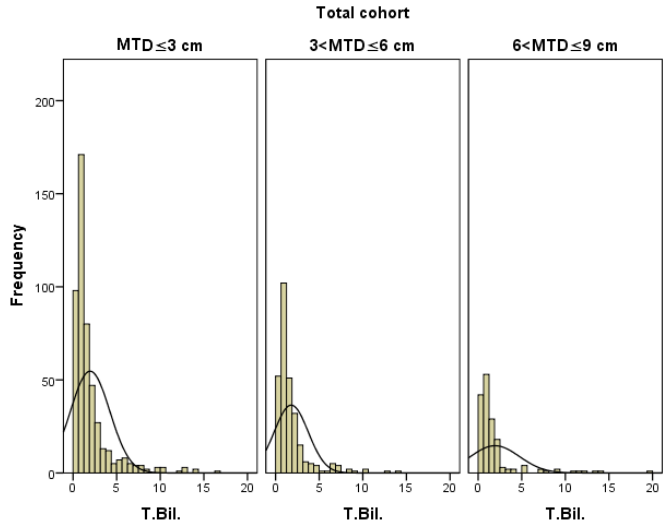
MTD tercile analysis for GGT levels in patients in the total cohort.

	MTD≤3 cm		3<MTD≤6 cm		6<MTD≤9 cm		
B.	n	Median (min.max.)	n	Median (min.max.)	n	Median (min.max.)	p
GGT	496	71.5 (8-804)[a]	285	74 (11-912)[a]	163	95.5 (13-858)[b]	0.025



MTD tercile analysis for AST levels in patients in the total cohort.

	MTD≤3 cm		3<MTD≤6 cm		6<MTD≤9 cm		
C.	n	Median (min. max.)	n	Median (min. max.)	n	Median (min. max.)	p
AST	496	41 (9-489)[a]	285	44 (12-334)[b]	163	46.5 (10-313)[a,b]	0.031

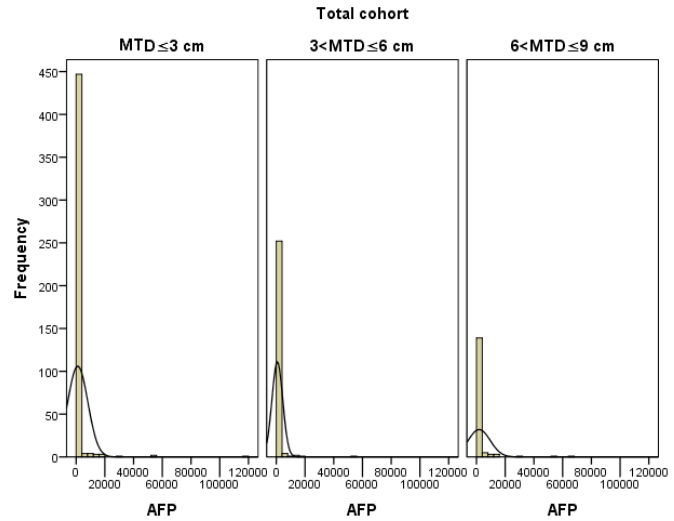


MTD tercile analysis for total bilirubin levels in patients in the total cohort.

	MTD≤3 cm		3<MTD≤6 cm		6<MTD≤9 cm		
D.	n	Median (min.max.)	n	Median (min.max.)	n	Median (min.max.)	p
T.Bil.	496	1.15 (0.13-16.4)	285	1.13 (0.21-13. 94)	163	1.0 (0.28-19.6)	0.191

MTD tercile analysis for albumin levels in patients in the total cohort.

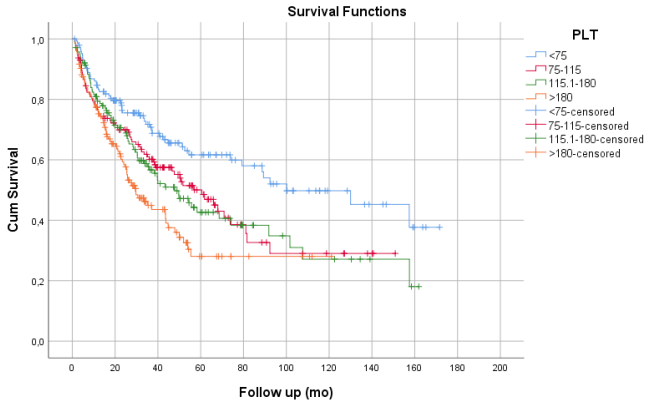
	MTD≤3 cm		3<MTD≤6 cm		6<MTD≤9 cm		
E.	n	Median (min.max.)	n	Median (min.max.)	n	Median (min.max.)	p
Alb.	496	3.1 (0.9-5.2)	285	3.2 (1-5.2)	163	3.4 (1-5.2)	0.238

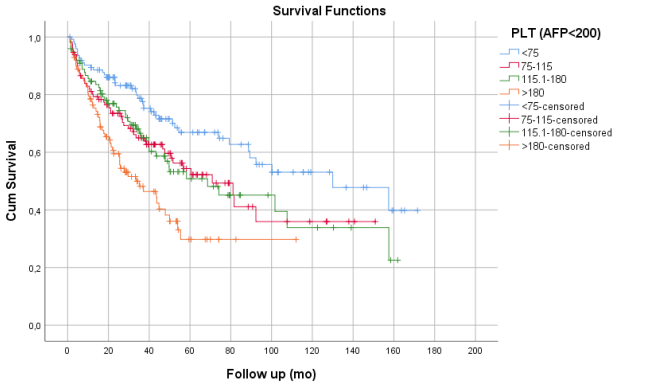


MTD tercile analysis for AFP levels in patients in the total cohort.

	MTD≤3 cm		3<MTD≤6 cm		6<MTD≤9 cm		
F.	n	Median (min.max.)	n	Median (min.max.)	n	Median (min. max.)	p
AFP	465	12.14 (0.2-118883)	261	17.4 (0.2-52891)	163	31 (0.1-66313.5)	0.170

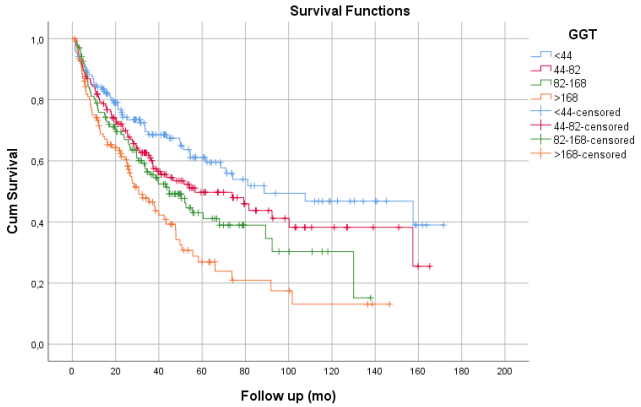
Abbreviations: MTD: Maximum Tumor Diameter; PLT: Platelets; GGT: Gamma Glutamyl Transferase; AST: Aspartate Amino Transferase; Alb: Albumin; T Bil: Total Bilirubin; AFP: Alpha-Fetoprotein. The Difference Between The Groups With Different Superscript Letters A, B Were Found To Be Statistically Significant.

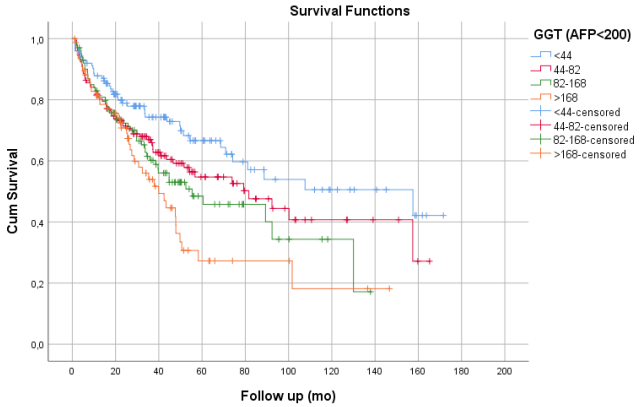




A.PLT	Survival (mo.) Mean±SE	Survival (mo.) Median±SE	Log-Rank p-value
<75	101.36±7.02	100.2±21.63	<0.001
75-115	69.89±6.22	60.5±8.05	
115.1-180	71.47±6.74	47.9±7.44	
>180	50.42±4.79	29.7±4.26	

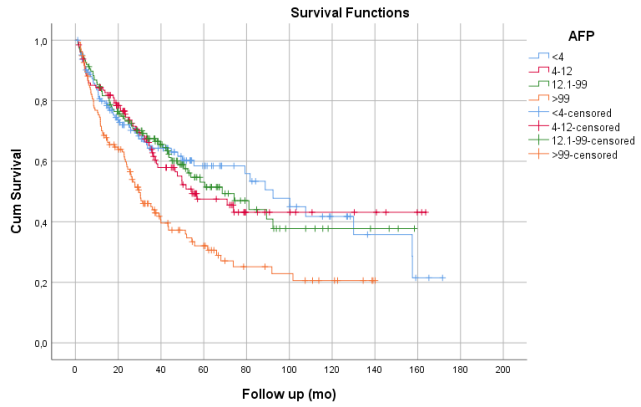
D.PLT (AFP<200)	Survival (mo.) Mean±SE	Survival (mo.) Median±SE	Log-Rank p-value
<75	108.52±7.48	130.0±31.31	<0.001
75-115	78.99±7.40	70.9±11.87	
115.1-180	82.53±8.21	68.6±21.08	
>180	49.91±5.22	33.5±8.67	





B.GGT	Survival (mo.) Mean±SE	Survival (mo.) Median±SE	Log-Rank p-value
<44	99.17±7.19	88.7±29.49	<0.001
44-82	82.94±6.51	56.7±15.68	
82.1-168	63.76±5.54	44.8±7.20	
>168	47.93±5.58	30.7±4.82	

E.GGT (AFP<200)	Survival (mo.) Mean±SE	Survival (mo.) Median±SE	Log-Rank p-value
<44	106.63±7.92	157.5±49.23	<0.001
44-82	88.08±7.25	81.6±18.60	
82.1-168	70.03±6.53	55.4±18.20	
>168	56.24±7.72	40.0±6.42	



C.AFP	Survival (mo.) Mean±SE	Survival (mo.) Median±SE	Log-Rank p-value
<4	90.41±7.33	92.4±11.94	<0.001
4-12	87.06±7.33	54.5±11.60	
12.1-99	83.39±6.84	68.6±12.51	
>99	51.89±5.03	29.7±3.92	

Abbreviations: as in Figure 1. AFP <200 means patients with AFP levels <200 IU/mL.

Discussion

Peripheral blood platelet counts are intimately related to hepatocarcingenesis and carry a uniquely pleiotropic role in defining the prognosis of patients with HCC.

On the one hand, as a hallmark of portal hypertension, thrombocytopenia identifies patients with a higher risk of decompensation from underlying chronic liver disease. Conversely, HCC patients with thrombocytosis carry a worse prognostic outlook due to the cancer related pro-inflammatory diathesis that accompanies abnormal thrombocytopoiesis and identifies patients with faster pace of tumour progression.

In this study, we found that blood platelet levels relate significantly to tumor size (MTD) and to survival (Figures 1 and 2). Both of these findings also help in diagnosis and management of patients with low blood AFP levels, a large group of patients for whom useful biomarkers are largely missing. Blood AST levels also significantly related to MTD and survival, as did blood

Table 1: Clinical characteristics of HCC patients (n=944) dichotomized by blood platelet levels.

		PLT<125 x10^9/L		PLT≥125 x10^9/L		
		n	Median (min.-max.)	n	Median (min.-max.)	p
MTD≤6 cm	PLT x10^9/L	387	78 (2.64-124)	394	210 (125-920)	<0.001
	MTD (cm)	387	4.2 (3.1-6)	394	5 (3.2-6.3)	0.021
	AFP IU/mL	373	12.14 (0.3-55000)	353	18.6 (0.2-118883)	0.103
	T.Bil. mg/dL	387	1.7 (0.13-13.94)	394	0.88 (0.2-16.4)	<0.001
	AST IU/mL	387	53 (9-489)	394	41 (10-340)	<0.001
	GGT IU/mL	387	68 (11-645)	394	82.5 (8-912)	0.051
	ALKP IU/mL	387	120 (24-827)	394	109.5 (3.3-980)	0.042
	Alb. g/dL	387	3 (1.2-5.2)	394	3.5 (0.9-5.1)	<0.001
	Hb g/dL	387	12.6 (6.3-17.2)	394	13 (7-18)	<0.001
	NE x10^9/L	385	2.79 (0.4-23.6)	394	4.36 (1-24.69)	<0.001
	LY x10^9/L	385	1.08 (0.18-18)	393	1.6 (0.3-8.5)	<0.001
		n	%	n	%	p
	Multifocality	187	48.3	185	47.0	0.702
	PVT (+)	72	19.7	114	31.8	<0.001
	Survival (mo.)	n	Mean±SE	n	Mean±SE	p
	Surgery	192	99.72±5.83	86	84.29±8.46	0.175
	No-surgery	59	21.24±5.91	83	27.70±4.50	0.329
		n	Median (min.-max.)	n	Median (min.-max.)	p
6<MTD≤9 cm	PLT x10^9/L	59	93 (26-124)	104	223.5 (125-653)	<0.001
	MTD (cm)	59	7.5 (6.3-9)	104	8 (6.2-9)	0.312
	AFP IU/mL	57	25 (1-55000)	95	37 (0.1-66313.5)	0.788
	T.Bil. mg/dL	59	1.6 (0.38-13.9)	104	0.8 (0.28-19.6)	<0.001
	AST IU/mL	59	67 (22-275)	104	37 (10-313)	<0.001
	GGT IU/mL	59	89 (13-858)	104	98 (14-684)	0.752
	ALKP IU/mL	59	121 (28-536)	104	128 (18-567)	0.579
	Alb. g/dL	59	3 (1-4.2)	104	3.5 (1-5.2)	<0.001
	Hb g/dL	59	13 (7-17.2)	104	14 (7.9-18)	0.146
	NE x10^9/L	59	3.5 (1.07-12.36)	103	4.75 (1-11.25)	0.851
	LY x10^9/L	58	1.04 (0.12-3.07)	102	1.6 (0.4-5.2)	<0.001
		n	%	n	%	p
	Multifocality	32	54.2	51	49.0	0.523
	PVT (+)	14	25.0	26	28.6	0.778
	Survival (mo)	n	Mean±SE	n	Mean±SE	p
	Surgery	24	44.27±10.53	12	49.58±17.85	0.795
	No-surgery	4	19.74±2.57	16	14.28±4.70	0.360

Abbreviations: T.Bil: Total Bilirubin; MTD: Maximum Tumor Diameter; PVT: Portal Vein Thrombosis; AFP: Alpha-Fetoprotein; T. Bil: Total Bilirubin; AST: Aspartate Amino Transferase; ALKP: Alkaline Phosphatase; GGT: Gamma Glutamyl Transferase; PLT: Platelets; Alb: Albumin; Hb: Hemoglobin; NE: Neutrophils; LY: Lymphocytes. Survival Analyses Are By The Kaplan-Meier Method.

Table 2A: Clinical characteristics of HCC patients (n=944) dichotomized by blood platelet levels.

		Univariable Logistic Regression		**Multivariable Logistic Regression**	
		OR (95% C.I)	**p**	**OR (95% C.I)**	**p**
Gender	**Female**	**reference**			
	Male	**0.787 (0.554-1.118)**	**0.182**		
Number of tumors	<2	reference			
	≥2	1.165 (0.886-1.532)	0.276		

PLT	<125	reference		reference	
	≥125	2.145 (1.605-2.867)	<0.001	1.887 (1.315-2.708)	0.001
AFP	<20	reference		reference	
	≥20	1.328 (1.002-1.760)	0.049	1.569 (1.119-2.202)	0.009
GGT	<60	reference			
	≥60	1.411 (1.058-1.883)	0.019		
AST	<40	reference			
	≥40	0.842 (0.638-1.112)	0.226		
ALKP	<120	reference			
	≥120	1.344 (1.021-1.768)	0.035		
PVT	No	reference			
	Yes	0.980 (0.704-1.364)	0.903		
Age		1.001 (0.990-1.012)	0.878		
NE# (2.1 - 6.1)		1.070 (1.014-1.129)	0.014		
LY# (1.3 - 3.5)		1.004 (0.884-1.140)	0.952		
CRP		0.995 (0.970-1.021)	0.727		
ESR		1.005 (0.994-1.017)	0.373		
WBC (4.3 - 10.3)		1.097 (1.043-1.155)	<0.001		
Hb		1.117 (1.049-1.190)	0.001		
T.Bili. (0.5 - 1.2)		0.982 (0.923-1.046)	0.577		
Albumin (3.4 - 4.8)		1.273 (1.057-1.535)	0.011		
ALT (0 - 55)		1.000 (0.997-1.003)	0.931		
Cholesterol (0 - 199)		1.006 (1.003-1.009)	<0.001		
Triglyceride (0 - 149)		1.002 (1.000-1.005)	0.031		
VLDL		1.004 (0.996-1.011)	0.381		
LDL (0-100)		1.008 (1.004-1.012)	<0.001	1.005 (1.001-1.009)	0.013
HDL		1.000 (0.990-1.011)	0.941		

Abbreviations: T.Bil: Total Bilirubin; AFP: Alpha-Fetoprotein; AST: Aspartate Amino Transferase; ALKP: Alkaline Phosphatase; GGT: Gamma Glutamyl Transferase; PLT: Platelets; PVT: Portal Vein Thrombosis; NE: Neutrophils; LY: Lymphocytes; Hb: Hemoglobin; CRP: C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate; Hb: Hemoglobin; WBC: White Blood Count; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; VLDL: Very Low Density Lipoprotein.

Table 2B: Logistic regression analysis OR for MTD>6 cm, reference: MTD≤6 cm).

	Univariable Logistic Regression	
	OR (95% C.I)	p
PLT<125 & AFP<20 & GGT<60 & ALKP<120 (n=87)	reference	
PLT ≥125 & AFP≥20 & GGT≥60 & ALKP≥120 (n=133)	4.014 (2.027-7.947)	<0.001

Abbreviations: T.Bil: Total Bilirubin; AFP: Alpha-Fetoprotein; AST: Aspartate Amino Transferase; ALKP: Alkaline Phosphatase; GGT: Gamma Glutamyl Transferase; PLT: Platelets; PVT: Portal Vein Thrombosis; NE: Neutrophils; LY: Lymphocytes; Hb: Hemoglobin; CRP: C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate; Hb: Hemoglobin; WBC: White Blood Count; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; VLDL: Very Low Density Lipoprotein.

Table 3A: Cox regression analysis (for MTD≤6 cm). Survival>30 days.

		Univariable Cox Regression		**Multivariable Cox Regression**	
		HR (95% C.I)	**p**	**HR (95% C.I)**	**p**
Gender	**Female**	**reference**			
	Male	0.998 (0.680-1.465)	0.992		
HBV	No	reference			
	Yes	0.800 (0.600-1.066)	0.127		

Number of tumors	<2	reference			
	≥2	1.057 (0.799-1.399)	0.698		
PLT	<125	reference			
	≥125	1.613 (1.217-2.139)	0.001		
AFP	<20	reference			
	≥20	1.530 (1.148-2.038)	0.004		
GGT	<60	reference			
	≥60	1.753 (1.301-2.363)	<0.001	1.840 (1.060-3.195)	0.030
AST	<40	reference			
	≥40	0.906 (0.677-1.211)	0.504		
ALKP	<120	reference			
	≥120	1.275 (0.963-1.688)	0.089		
PVT	No	reference			
	Yes	1.769 (1.262-2.481)	0.001		
Age		1.031 (1.017-1.045)	<0.001		
NE# (2.1 - 6.1)		1.083 (1.033-1.135)	0.001		
LY# (1.3 - 3.5)		0.899 (0.759-1.064)	0.215		
CRP		1.033 (1.013-1.053)	0.001	1.034 (1.013-1.054)	0.001
ESR		1.005 (0.987-1.024)	0.556		
WBC (4.3 - 10.3)		1.072 (1.019-1.128)	0.008		
Hb		0.964 (0.911-1.019)	0.195		
T.Bili. (0.5 - 1.2)		0.914 (0.844-0.991)	0.028		
Albumin (3.4 - 4.8)		1.092 (0.895-1.332)	0.385		
Sodium (136 - 145)		1.018 (0.980-1.057)	0.364		
CRE		1.041 (1.017-1.066)	0.001		
ALT (0 - 55)		0.999 (0.996-1.002)	0.564		
Cholesterol (0 - 199)		1.002 (0.999-1.006)	0.198		
Triglyceride (0 - 149)		1.001 (0.999-1.004)	0.303		
VLDL		1.008 (0.995-1.022)	0.220		
LDL (0-100)		1.003 (0.998-1.007)	0.236		
HDL		0.994 (0.984-1.004)	0.234		

Abbreviations: T.Bil: Total Bilirubin; AFP: Alpha-Fetoprotein; AST: Aspartate Amino Transferase; ALKP: Alkaline Phosphatase; GGT: Gamma Glutamyl Transferase; PLT: Platelets; PVT: Portal Vein Thrombosis; NE: Neutrophils; LY: Lymphocytes; Hb: Hemoglobin; CRP: C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate; Hb: Hemoglobin; WBC: White Blood Count; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; VLDL: Very Low Density Lipoprotein.

Table 3B: Cox regression analysis (for MTD>6 cm). Survival>30 days.

		Univariable Cox Regression		Multivariable Cox Regression	
		HR (95% C.I)	**p**	**HR (95% C.I)**	**p**
Gender	Female	reference			
	Male	1.144 (0.562-2.326)	0.711		
HBV	No	reference			
	Yes	1.784 (1.068-2.982)	0.027		
Number of tumors	<2	reference			
	≥2	0.606 (0.363-1.012)	0.056		
PLT	<125	reference			
	≥125	1.125 (0.664-1.907)	0.661		

AFP	<20	reference			
	≥20	1.032 (0.619-1.721)	0.904		
GGT	<60	reference			
	≥60	1.544 (0.887-2.686)	0.124		
AST	<40	reference			
	≥40	0.802 (0.481-1.337)	0.397		
ALKP	<120	reference		reference	
	≥120	1.742 (1.046-2.901)	0.033	2.004 (1.039-3.863)	0.038
PVT	No	reference			
	Yes	0.983 (0.528-1.831)	0.957		
Age		1.017 (0.996-1.038)	0.111		
NE# (2.1 - 6.1)		1.065 (0.950-1.194)	0.279		
LY# (1.3 - 3.5)		0.884 (0.597-1.308)	0.536		
CRP		1.027 (0.994-1.062)	0.108		
ESR		1.016 (0.994-1.039)	0.144		
WBC (4.3 - 10.3)		1.052 (0.952-1.163)	0.322		
Hb		0.856 (0.760-0.965)	0.011		
T.Bili. (0.5 - 1.2)		1.012 (0.909-1.128)	0.822		
Albumin (3.4 - 4.8)		0.716 (0.503-1.019)	0.064		
Sodium (136 - 145)		0.913 (0.852-0.978)	0.009		
CRE		2.478 (1.384-4.436)	0.002	3.016 (1.405-6.473)	0.005
ALT (0 - 55)		1.002 (0.995-1.008)	0.615		
Cholesterol (0 - 199)		1.000 (0.995-1.006)	0.906		
Triglyceride (0 - 149)		1.002 (0.997-1.006)	0.418		
VLDL		1.007 (0.980-1.035)	0.601		
LDL (0-100)		1.001 (0.995-1.007)	0.778		
HDL		0.972 (0.949-0.995)	0.018		

Abbreviations: T.Bil: Total Bilirubin; AFP: Alpha-Fetoprotein; AST: Aspartate Amino Transferase; ALKP: Alkaline Phosphatase; GGT: Gamma Glutamyl Transferase; PLT: Platelets; PVT: Portal Vein Thrombosis; NE: Neutrophils; LY: Lymphocytes; Hb: Hemoglobin; CRP: C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate; Hb: Hemoglobin; WBC: White Blood Count; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; VLDL: Very Low Density Lipoprotein.

Table 3C: Cox regression analysis for combined parameters. Survival>30 days.

			Univariable Cox Regression	
			HR (95% C.I)	**p**
MTD ≤ 6 cm	total	PLT<125 & AFP<20 & GGT<60	reference	
		PLT≥125 & AFP≥20 & GGT≥60	4.000 (2.343-6.828)	<0.001
MTD ≤ 6 cm	PVT+	PLT<125 & AFP<20 & GGT<60	reference	
		PLT≥125 & AFP≥20 & GGT≥60	3.545 (1.146-10.964)	0.028
MTD ≤ 6 cm	PVT-	PLT<125 & AFP<20 & GGT<60	reference	
		PLT≥125 & AFP≥20 & GGT≥60	4.185 (2.163-8.098)	<0.001
MTD > 6 cm	total	ALKP<120 & ALB≥3.5	reference	
		ALKP≥120 & ALB<3.5	2.543 (1.268-5.100)	0.009
MTD > 6 cm	PVT-	ALKP<120 & ALB≥3.5	reference	
		ALKP≥120 & ALB<3.5	2.583 (1.204-5.539)	0.015

Abbreviations: MTD: Maximum Tumor Diameter; AFP: Alpha-Fetoprotein Iu/Ml; GGT: Gamma Glutamyl Transferase IU/Ml; ALKP: Alkaline Phosphatase IU/Ml; Alb: Albumin G/Dl; PLT: Platelets $X10^9$/L; PVT: Portal Vein Thrombosis.

total albumin levels, as noted previously [14,15]. It has been thought that increasing HCC growth would displace or destroy liver parenchyma, with resulting increase in bilirubin levels and decrease in albumin levels [16]. However, that was not the finding here. We found that a decrease in platelet levels was accompanied by an increase in bilirubin and AST and a decrease in albumin levels, all consistent with cirrhotic portal hypertension (Table 1). By contrast, the highest platelet levels were accompanied by normal bilirubin levels, normal (higher) albumin levels and larger MTD tumors. How might this be best explained? One possibility is that these HCCs become large through rapid growth, before portal hypertension can develop. A second possibility is that they grow very slowly for a long time and the liver parenchyma can adapt to the growing tumor without hepatic decompensation. A third possibility is that there are several different pathways to the development of large HCCs, perhaps one associated with inflammation and low platelets and another not inflammation-associated.

Furthermore, the precursors of large HCCs with and without thrombocytopenia are likely to be different, since thrombocytopenia is usually irreversible without therapy. Thus, small HCCs with thrombocytopenia can be surmised to not be able to grow into large HCCs without thrombocytopenia. By parallel argument, large HCCs with normal platelets and absent or mimimal cirrhosis are likely to have precursors with normal platelets. Maybe there are also slow-growing small HCCs that never become large, but since they are usually treated when found, it may be difficult to find evidence for that. For the patients with MTD ≤6 cm there was a significantly higher percentage with PVT in the non thrombocytopenia-associated compared with the thrombocytopenia-associated group. This difference in percentage of PVT patients disappeared in the large MTD patient group.

Thrombocytopenia has been previously observed as a predictor of HCC development [7,17] and has also been found to be related to small HCC size [9]. Conversely, thrombocytosis has been found to be associated with large HCCs [18,19,20]. Our regression analysis found several significant factors that significantly related to large HCC size (Table 2A), with the highest odds ratios (OR) for HCCs >6cm being levels of platelets (OR 2.154), GGT (OR 1.411), ALKP (OR 1.344) and AFP (OR 1.328). A model combining elevated levels of all 4 parameters gave the highest odds ratio of 4.014 for prediction of large size >6cm HCCs (Table 2B).

Cumulative and median survivals (Figure 2) had a significant correlation with levels of platelets, GGT and AFP in the total cohort, and also had a significant correlation with levels of platelets and GGT in patients with low AFP levels (<200 IU/mL). A Cox regression analysis on death was performed separately on patients with ≤6cm MTD and >6cm MTD (Table 4). Parameters with hazard ratios >1.5 that were also statistically significant were platelets, AFP, GGT and presence of PVT in the smaller MTD category (Table 3A). However, they were different for the larger MTD category (Table 3B), being presence of HBV, AFP, ALKP and albumin (protective, hazard ratio of 0.716). A combination of parameters with platelets, AFP and GGT together gave a hazard ratio of 4.0 in the total small MTD category (Table 3C). The hazard ratio was very similar when patients with or without PVT were analyzed separately. A combination of ALKP and albumin was used for the large MTD category and produced a hazard ratio of 2.543 (Table 3C). However insufficient patient numbers precluded separate analysis for patients with or without PVT in patients with large tumors. Thus, survival factors were different for HCC patients having small or large MTDs.

In this study, blood platelet numbers were used as a cirrhosis surrogate, leading to the assignment of HCC patients to 2 clinical groups, based upon presence or absence of portal hypertension. This in turn led to the observation of differences in MTD and percent of patients with PVT (Table 1). For patients with small HCCs, the ratio of those with high or low platelets was essentially 1:1. However for patients with large HCCs, the ratios were 1:2 (Table 1). Presumably patients with large HCCs and normal platelet levels could only derive from small HCCs with normal platelet levels.

We speculate that the causes of death in the 2 groups of patients with larger tumors may be different. They are likely due to liver failure in HCC patients with low platelets and portal hypertension, but due to cancer death in HCC patients with normal platelets. This is given some support from the differences in tumor factors (MTD, AFP and PVT) in patients with thrombocytopenia compared to those without it (Table 1). In addition, patients with cirrhosis typically have inflammation in their microenvironment [21] that may be important in HCC growth and aggressive biology in these patients. Their higher AST levels (Table 1) suggests this. By contrast, patients with normal platelets probably have different drivers of their HCCs, that might include oncogenes, platelet-derived growth factors, or other non inflammation-associated tumor factors.

Although blood platelet levels were used in this work only as a surrogate for cirrhosis, they could also play a role in the HCC aggressiveness differences between patients without portal hypertension (normal platelets) and patients with portal hypertension (thrombocytopenia). Platelets are involved in the thrombosis that has long been known to be increased in patients with a variety of cancer types [22,23], and thrombocytosis is well described as being associated with an aggressive phenotype in several cancer types [24,25,26]. Furthermore, platelet alpha granules produce a varienty of factors that are known to be involved in HCC growth [27], including Platelet-Derived Growth Factor (PDGF), Epidermal Growth Factor (EGF), Vascular Endothelial Growth Factor (VEGF), Hepatocyte Growth Factor (HGF), Insulin-Like Growth Factor (IGF) and several inflammatory cytokines, in addition to P-selectin, which is involved in thrombosis [28]. Since there are several clinically-available inhibitors of these growth factors, they might have a role in future HCC therapy. Furthermore, the anti-platelet inhibitors aspirin and clopidogrel have potential for therapy of HCC patients with normal platelets (but not those with low platelets, who are at risk of bleeding). Epidemiological studies have already suggested that aspirin use may be associated with lower incidence of HCC [29-31]. Conversely, platelet lysates can directly stimulate HCC cell growth [32].

However, this hypothesis, which focuses on the role of cirrhosis and portal hypertension in HCC phenotypes could be a consequence and not a cause. In this opposite view, some HCCs may have much faster growth rates than others and could produce large size HCCs before portal hypertension has time to develop. If true, then large HCCs without portal hypertension might be expected to have higher indices of growth, such as Ki-67 index, than HCCs of similar size that have portal hypertension. Another prediction of these ideas is that pathological study of portal micro-invasion by HCC, microsatellite instability or tumor mutation burden might positively correlate with peripheral blood platelet levels or other indices of portal hyper-

tension. This study is also limited by its relatively small sample size, since we could not separately test our survival model on patients with large HCCs in presence of PVT (Table 3C). Nevertheless, this approach allows one to focus on possible different HCC development pathways in future in patients with and without portal hypertension and consider the possible therapeutic use of platelet inhibitors in selected HCC sub-groups.

Abbreviations: HCC: Hepatocellular Carcinoma; WBC: White Blood Count; Tbil: Total Bilirubin; AST: Aspartate Amino Transferase; ALT: Alanine Aminotransferase; ALKP: Alkaline Phosphatase; GGT: Gamma Glutamyl Transferase; MTD: Maximum Tumor Diameter; PVT: Portal Vein Thrombosis; AFP: Alpha-Fetoprotein; CAT Scan: Computed Axial Tomography; OR: Odds Ratio; HR: Hazard Ratio; HBV: Hepatitis B Virus; CRP: C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate; PDGF: Platelet-Derived Growth Factor; EGF: Epidermal Growth Factor; VEGF: Vascular Endothelial Growth Factor; HGF: Hepatocyte Growth Factor; LGF: Insulin-Like Growth Factor.

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