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Research Article

# Erythrocyte Sedimentation Rate and C-Reactive Protein are Markers for Tumor Aggressiveness and Survival in Patients with Hepatocellular Carcinoma

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## ABSTRACT

**Introduction:** Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are acute phase reactants in clinical use for monitoring inflammatory diseases for several decades. CRP is also prognostically useful in several cancers.

**Objective:** To evaluate the role of ESR as a possible indicator of tumor biology and survival in patients with hepatocellular carcinoma (HCC).

**Methods:** A large cohort of HCC patients in Turkey was examined retrospectively for clinical and tumor characteristics with respect to blood CRP and ESR levels.

**Results:** Portal vein thrombosis and high Aggressiveness Index were significantly related to elevated CRP or ESR levels and especially to the combination of elevated CRP and ESR, both in the total cohort and in patients with small tumors <5 cm. A final logistic regression model of an Aggressiveness Index score gave an Odds Ratio of 10.37 for the ESR and CRP combination, compared to the reference category. Furthermore, a Cox regression model on death gave a Hazard Ratio of 2.53 for the ESR and CRP combination versus the reference category for each of them (P<0.001). A significant Hazard Ratio for the ESR and CRP combination was also found for patients with low alpha-fetoprotein.

**Conclusions:** ESR is a useful biomarker for HCC extent and survival, especially in combination with CRP, in patients with small or large tumors and with elevated or low serum alpha-fetoprotein.

**Keywords:** HCC: Hepatocellular carcinoma; CRP: C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate; PVT: Portal Vein Thrombosis; MTD: Maximum Tumor Diameter

## ABBREVIATIONS

HCC: Hepatocellular carcinoma; PVT: Portal Vein Thrombosis; AFP: Alpha-Fetoprotein; GGTP: Gamma GlutamylTranspeptidase; ALKP: Alkaline Phosphatase; CRP: C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate; MTD: Maximum Tumor Diameter; CT: Computerized Axial Tomography; MRI: Magnetic Resonance Imaging

## INTRODUCTION

The acute phase reactants C-reactive protein (CRP) and Erythrocyte Sedimentation Rate (ESR) are cheap and readily available clinical laboratory tests, that are non-specific indices of inflammatory activity and have been in clinical use for several decades [1-4]. Blood levels of CRP have recently been found to be useful as markers both of disease extent and prognosis of

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several cancers, especially of the GI tract and for hepatocellular carcinoma (HCC) [5,6]. CRP is synthesized in the liver and in HCC cells [2,7,8] and is thought to reflect both systemic and local inflammation. Serum CRP may be elevated in HCC patients with either high or low serum alpha-fetoprotein (AFP) levels [8]. By contrast, ESR is not synthesized by any organ, but represents a change in blood viscosity due to elevated blood fibrinogen and other proteins that result in increased red cell rouleaux formation [9]. Unlike CRP, there have been few investigations of ESR as a predictor of solid tumor behavior [10], although it is a useful non-specific determinant of inflammatory activity of several diseases [11]. We and others have found that CRP is a useful indicator of HCC tumor aggressiveness factors, such as extent of maximum tumor diameter (MTD), level of serum alpha-fetoprotein (AFP), percent of patients with portal vein thrombosis (PVT) and an aggregate index of the sum of these tumor parameters together called an Aggressiveness Index [12-15], as well as for prognosis[16,17]. Here, we extend our previous work, by evaluating the relationship of ESR to HCC patient clinical and tumor characteristics and survival, both as a single parameter and especially in combination with CRP. This combination is a much more powerful predictor of HCC disease extent than CRP alone.

## METHODS

### Patient data

We analyzed a database of 1194 prospectively-accrued HCC patients who had full baseline tumor parameter data, including CT scan information on tumor size, number of tumor nodules, presence or absence of PVT, serum AFP levels; serum ESR and blood CRP measures; complete blood count; blood liver function tests, (total bilirubin, GGTP, ALKP, albumin, transaminases) and patient demographics. Diagnosis was made either via tumor biopsy or according to international guidelines. Database management conformed to legislation on privacy and this study conforms to the ethical guidelines of the Declaration of Helsinki. Approval for this retrospective study on de-identified HCC patients was obtained from the Institutional Review Board.

An Aggressiveness Index was calculated as the sum of scores for for MTD+AFP+PVT+# Nodules [12,13] MTD (cm), in tertiles: MTD<4.5; 4.5 ≤ MTD ≤ 9.6; MTD>9.6; scores 1, 2, 3 respectively; AFP IU/ml (cut-off): AFP<100; 100 ≤ AFP ≤ 1000; AFP>1000; scores 1, 2, 3 respectively; PVT: PVT(No); PVT(Yes); scores 1, 3 respectively; Number of Tumor Nodules: Nodules ≤ 3; Nodules>3; scores 1, 3 respectively.

Statistical analysis Mean and SD for continuous variables, and relative frequency for categorical variables, were used as indices of centrality and dispersion of the distribution. For categorical variables, the Chi-square and z test for proportions were used. The Wilcoxon rank-sum (Mann-Whitney) test was to test the difference between two categories. Logistic regression model was used to evaluate the associations between Aggressiveness Index Score (>4 vs. ≤ 4) and relative parameters. CRP cutoff of 6 mg/dL was determined by ROC analysis [13] and ESR cutoff was determined by upper limit of normal values in our clinical laboratories of 15 mm/hr (10 mm/hr for males, 18 mm/hr for females) and was identical to that reported in multivariate analysis [18,19].

Final multiple logistic regression models were obtained with the backward stepwise method and the variables that showed associations with P<0.10 were left in the models.

Survival information was available for 845 patients. Cox's Model was fitted to the data. The proportional hazard assumption was evaluated by means of Schoenfeld residuals. Model fitting was evaluated by means of Akaike Information Criteria and Bayesian Information Criterion. Risk estimators are expressed as Hazard Ratios (HR) and 95% Confidence Interval (95%CI). All variables were examined as categorical.

When testing the null hypothesis of no association, the probability level of α error, two tailed, was 0.05. All the statistical computations were made using STATA 16, Stata Corp. 2019. Stata Statistical Software: Release 16. College Station, TX: Stata Corp LLC.

## RESULTS

### ESR and CRP, separately and in combination, and tumor characteristics

We have previously shown linearity between serum C-reactive protein (CRP) and serum alpha-fetoprotein (AFP), MTD and Tumor Aggressiveness index [12,13]. As a next step, we compared the clinical and tumor features of patients with HCC dichotomized according to high or low serum CRP or high or low blood ESR in the total patient cohort (Table 1). The ESR dichotomization showed significantly higher AFP, percent of patients with PVT and Aggressiveness score (and higher MTD, P=0.07) for patients with higher ESR levels. The CRP dichotomization showed significantly higher MTD, Aggressiveness score and percent patients with PVT (but not AFP) in the patients with higher CRP levels. Serum albumin levels were lower (normal) and GGTP levels were higher in both the elevated ESR and the CRP groups, compared to the lower levels of each. ESR and CRP levels were then considered together. As shown in the lower part of Table 1, patients with higher levels of the combination of these 2 parameters had significantly higher MTD, percent with PVT and Aggressiveness index, but not significantly higher AFP levels.

| | ESR (mm/hr) | | | CRP (mg/L) |
|---|---|---|---|---|
| Variables* | ≤ 30 | >30 | p ψ | ≤ 6 (mg/L) |
| Albumin (g/dL) | 4.90 ± 12.63 | 3.77 ± 8.92 | 0.01 | 3.35 ± 1.28 |
| ALKP (U/L) | 173.25 ± 172.08 | 205.02 ± 208.50 | 0.005 | 168.44 ± 180.11 |

| | | | | | | |
|---|---|---|---|---|---|---|
| GGTP (U/L) | 113.51 ± 116.23 | 165.38 ± 195.97 | 0.002 | | 129.01 ± 148.06 | |
| Total Bilirubin (mg/dL) | 2.40 ± .02 | 2.50 ± 3.73 | 0.36 | | 2.22 ± 3.41 | |
| MTD (cm) | 5.67 ± 4.14 | 6.16 ± 3.89 | 0.07 | | 5.41 ± 3.69 | |
| PVT (% +ve) | 22.31 | 34.33 | 0.005 ^ | | 24.06 | |
| AFP (IU/mL) | 4193.60 ± 22750.2 | 6713.14 ± 43541.3 | 0.04 | | 5956.00 ± 38983.7 | |
| Aggressiveness Index score | 6.18 ± 2.06 | 6.75 ± 1.99 | 0.001 | | 6.24 ± 1.97 | |
| | | | | | | |
| | ESR and CRP combined | | | | | |
| | ESR ≤ 30 and CRP≤6 | | | | ESR>30 and CRP>6 | |
| | | | | | | |
| MTD (cm) | 5.04±3.39 | | | | 6.55±3.59 | |
| PVT (% +ve) | 20.11 | | | | 42.86 | |
| AFP (IU/mL) | 4083.22 ± 23296.96 | | | | 3441.83 ± 12238.46 | |
| Aggressiveness Index | 6.06 ± 2.00 | | | | 7.22 ± 2.07 | |

All values: Means±Standard Deviation as continuous; Frequencies and Percentage (%) as categorical; ψ Wilcoxon rank-sum (Mann-Whitney) test; ^ Chi-square test.

Abbreviations: ESR: Erythrocyte Sedimentation Rate ESR (mm/hr); CRP: C-Reactive Protein (mg/dL); MTD: Maximum Tumor Diameter; AFP: Alpha-Fetoprotein; PVT: Portal Vein Thrombosis; Alkalinephosphatase; GGTP: Gamma Glutamyltranspeptidase

Aggressiveness Index as sum of scores (Ref 16):

MTD (in terciles): MTD<4.5; 4.5 ≤ MTD≤9.6; MTD>9.6; scores 1, 2, 3 respectively;

AFP (cut-off): AFP<100; 100 ≤ AFP ≤ 1000; AFP>1000 ng/ml; scores 1, 2, 3 respectively;

PVT (No/Yes): PVT (No); PVT (Yes); scores 1, 3 respectively;

Tumor Nodules (number): Nodules ≤ 3; Nodules>3; scores 1, 3 respectively.

**Table 1:** HCC patient characteristics of total cohort, in ESR (≤ 30/>30), CRP (≤ 6/>6 mg/L) and combined categories.

## ESR and CRP, separately and in combination in different tumor size groups

ESR or CRP dichotomizations were next evaluated separately in patients according to MTD<5 cm or 5>MTD<10 cm (Table 2). For the smaller tumors, the high ESR group was significantly greater than the low ESR group only for AFP and Aggressiveness score, but not for MTD or percent patients with PVT. The high and low CRP groups did not discriminate between any tumor characteristics. In comparison to the patients with smaller tumors, patients with larger tumors had significantly greater MTD, AFP, percent PVT and Aggressiveness index in patients in the high versus low ESR group. By contrast, the CRP dichotomization could not distinguish patient tumor characteristics, based on their CRP levels. Thus, ESR could distinguish between tumor characteristics in both smaller and larger MTD patients. For the important small <5 cm MTD patients, ESR plus CRP were then considered together (Table 3). Patients in the high combination group had significantly higher percent PVT and Aggressiveness score than patients in the low combination group, higher but not significant AFP levels, P=0.07, and no significant differences in MTD.

| | **ESR (mm/hr)** | | | **CRP (mg/L)** |
|---|---|---|---|---|
| Variables * | ≤30 | >30 | p ψ | ≤ 6 |
| MTD<5 cm | | | | |
| Albumin (g/dL) | 5.43 ± 14.94 | 2.98 ± 1.22 | 0.0002 | 3.26 ± 0.76 |
| ALKP (U/L) | 133.93 ± 80.31 | 238.20 ± 271.37 | 0.0001 | 142.62 ± 172.75 |
| GGTP (U/L) | 115.44 ± 120.90 | 183.64 ± 233.98 | 0.0005 | 113.87 ± 155.71 |
| Total Bilirubin (mg/dL) | 2.08 ± 3.30 | 2.95 ± 3.92 | 0.02 | 2.07 ± 2.90 |
| MTD (cm) | 2.80 ± 1.11 | 3.01 ± 0.91 | 0.1 | 2.96 ± 1.13 |
| PVT (% +ve) | 15.58 | 21.15 | 0.17 ^ | 15.52 |
| AFP (IU/mL) | 1191.95 ± 11986.01 | 3271.83 ± 17192.96 | 0.003 | 2400.19 ± 15923.79 |
| Aggressiveness Index score | 5.13 ± 1.37 | 5.67 ± 1.53 | 0.006 | 5.26 ± 1.35 |

| | | | | |
|---|---|---|---|---|
| 5 > MTD<10 cm | | | | |
| Albumin (g/dL) | 3.34 ± 1.84 | 3.10 ± 1.52 | 0.1 | 3.54 ± 1.95 |
| ALKP (U/L) | 199.08 ± 200.61 | 236.34 ± 244.47 | 0.001 | 195.31 ± 202.32 |
| GGTP (U/L) | 138.79 ± 159.57 | 198.32 ± 206.03 | 0.002 | 138.77 ± 131.51 |
| Total Bilirubin (mg/dL) | 2.42 ± 3.82 | 2.63 ± 3.95 | 0.58 | 2.33 ± 4.08 |
| MTD (cm) | 6.57 ± 1.34 | 6.98 ± 1.52 | 0.05 | 6.61 ± 1.46 |
| PVT (% +ve) | 23.62 | 44.8 | <0.001^ | 26.61 |
| AFP (IU/mL) | 4736.72 ± 28422.47 | 8544.48 ± 55177.01 | 0.05 | 8642.87 ± 58873.46 |
| Aggressiveness Index score | 6.67 ± 1.79 | 7.14 ± 1.64 | 0.05 | 6.91 ± 1.73 |

* All values: Means ± Standard Deviation as continuous; Frequencies and Percentage (%) as categorical; ψ Wilcoxon rank-sum (Mann-Whitney) test; ^ Chi-square test.

Abbreviations: MTD: Maximum Tumor Diameter; ALKP: Alkaline Phosphatase; GGTP: Gamma Glutamyltranspeptidase; AFP: Alpha-Fetoprotein; PVT: Portal Vein Thrombosis; ESR: Erythrocyte Sedimentation Rate; CRP: C-Reactive Protein.

The Aggressiveness Index (Ref 16) is the sum of the scores for MTD +AFP+ PVT+# Nodules.

Thus: MTD (in tertiles): MTD<4.5; 4.5 ≤ MTD ≤ 9.6; MTD>9.6; scores 1, 2, 3 respectively.

AFP (cut-off): AFP<100; 100 ≤ AFP ≤ 1000; AFP>1000 ng/ml; scores 1, 2, 3 respectively.

PVT (No/Yes): PVT (No); PVT(Yes); scores 1, 3 respectively. Tumor Nodule (number): Nodules ≤ 3; Nodules>3; scores 1, 3 respectively.

**Table 2:** HCC patient characteristics in defined MTD groups: comparisons of ESR ( ≤ 30/>30) and CRP ( ≤ 6/>6 mg/L) categories.

| | ESR(mm/hr) and CRP(mg/L) combined | |
|---|---|---|
| Variables * | ESR ≤ 30 and CRP ≤ 6 | ESR>30 and CRP>6 |
| MTD<5 cm | | |
| Albumin (g/dL) | 3.30 ± 0.77 | 3.28 ± 2.43 |
| ALKP (U/L) | 120.90 ± 73.48 | 281.77 ± 309.96 |
| GGTP (U/L) | 86.33 ± 95.53 | 221.23 ± 391.82 |
| Total Bilirubin (mg/dL) | 1.80 ± 2.30 | 5.40 ± 7.22 |
| MTD (cm) | 2.92 ± 1.19 | 2.97 ± 1.02 |
| PVT (% +ve) | 13.89 | 38.46 |
| AFP (IU/mL) | 249.64 ± 1098.92 | 2506.28 ± 6077.66 |
| Aggressiveness Index score | 5.18 ± 1.40 | 6.32 ± 1.93 |

* All values: Means ± Standard Deviation as continuous; Frequencies and Percentage (%) as categorical. ψ Wilcoxon rank-sum (Mann-Whitney) test; ^ Chi-square test.

Abbreviations: MTD: Maximum Tumor Diameter; ALKP: Alkaline Phosphatase; GGTP: Gamma Glutamyl Transpeptidase; AFP: Alpha-Fetoprotein; PVT: Portal Vein Thrombosis; ESR: Erythrocyte Sedimentation Rate; CRP: C-Reactive Protein

The Aggressiveness Index is the sum of the scores for MTD+AFP +PVT+# Nodules.

Thus: MTD (in tertiles): MTD<4.5; 4.5 ≤ MTD ≤ 9.6; MTD>9.6; scores 1, 2, 3 respectively.

AFP (cut-off): AFP<100; 100 ≤ AFP ≤ 1000; AFP>1000 ng/ml; scores 1, 2, 3 respectively.

PVT (No/Yes): PVT(No); PVT(Yes); scores 1, 3 respectively.

Tumor Nodule (number): Nodules ≤ 3; Nodules>3; scores 1, 3 respectively.

**Table 3:** Clinical characteristics of patients with HCCs under 5 cm, for ESR (≤ 30/>30) and CRP (≤ 6/>6 mg/L) together.

## Logistic regression modelling and survival

All parameters were then considered together in a logistic regression model of the Aggressiveness Index score, including ESR, CRP, ALKP, GGTP, AST and ALT (Table 4A). CRP was significant with an Odds Ratio (OR) of 3.66 compared to the reference value. However, when ESR and CRP were considered in combination, the model yielded an OR of 11.66 for ESR>30 and CRP>6, compared to ESR<30 and CRP<6 (Table 4A, middle section). A similar high OR was found for the final regression model on all parameters in the backward stepwise method, with an OR of 10.37 for the high category of ESR>30 and CRP>6 versus the reference category (Table 4B). Interestingly, ALKP was significantly different in all models for high category versus reference category.

We then used the data in a Cox regression model on death, considering ESR or CRP alone or together and found significant differences in the hazard ratios (HRs) for ESR alone, CRP alone and ESR plus CRP combined. The HRs were: ESR alone 1.45 compared to 1.0 for reference, CRP alone 1.60 compared to 1.0 for reference, and ESR and CRP together had an HR of 2.53 versus the reference category (Table 5A). The Cox regression model was also calculated for a patient cohort with

low serum AFP (<100 IU/mL) values, for ESR alone, CRP alone or the combination of ESR and CRP (Table 5B), and the HRs were 1.27, 1.81 and 3.43, respectively, compared to HR of 1 for each reference category.

| Parameter | OR | Se(OR) | P | 95% C.I. |
|---|---|---|---|---|
| A) | | | | |
| ESR (mm/hr) | | | | |
| ≤ 30 (Ref. category) | 1 | | | |
| >30 | 1.36 | 0.4 | 0.29 | 0.76 to 2.43 |
| CRP (mg/L) | | | | |
| ≤ 6 (Ref. category) | 1 | | | |
| >6 | 3.66 | 1.8 | 0.008 | 1.40 to 9.59 |
| ALKP (U/L) | | | | |
| ≤ 200 (Ref. category) | 1 | | | |
| >200 | 2.87 | 1.29 | 0.02 | 1.18 to 6.95 |
| GGTP (U/L) | | | | |
| ≤ 200 (Ref. category) | 1 | | | |
| >200 | 1.69 | 0.79 | 0.26 | 0.67 to 4.25 |
| AST (U/L) | | | | |
| ≤ 40 (Ref. category) | 1 | | | |
| >40 | 0.9 | 0.3 | 0.76 | 0.46 to 1.75 |
| ALT (U/L) | | | | |
| ≤ 60 (Ref. category) | 1 | | | |
| >60 | 0.76 | 0.26 | 0.42 | 0.39 to 1.49 |
| | | | | |
| | | | | |
| ESR (mm/hr) and CRP(mg/L) combined | | | | |
| ESR ≤ 30 and CRP ≤ 6 (Ref. category) | 1 | | | |
| ESR ≤ 30 and CRP>6 | 2.16 | 1.24 | 0.18 | 0.70 to 6.66 |
| ESR >30 and CRP ≤ 6 | 1.19 | 0.37 | 0.57 | 0.65 to 2.18 |
| ESR>30&CRP>6 | 11.66 | 12.03 | 0.02 | 1.54 to 88.08 |
| ALKP (U/L) | | | | |
| ≤ 200 (Ref. category) | 1 | | | |
| >200 | 3.02 | 1.37 | 0.01 | 1.24 to 7.34 |
| GGTP (U/L) | | | | |
| ≤ 200 (Ref. category) | 1 | | | |
| >200 | 1.66 | 0.78 | 0.28 | 0.66 to 4.16 |
| AST (U/L) | | | | |
| ≤ 40 (Ref. category) | 1 | | | |
| >40 | 0.92 | 0.31 | 0.8 | 0.47 to 1.78 |
| ALT (U/L) | | | | |
| ≤ 60 (Ref. category) | 1 | | | |
| >60 | 0.76 | 0.26 | 0.43 | 0.39 to 1.50 |
| B) | | | | |
| ESR (mm/hr) and CRP(mg/L) combined | | | | |
| ESR ≤ 30 and CRP ≤ 6 (Ref. category) | 1 | | | |
| ESR>30 and CRP>6 | 10.37 | 10.61 | 0.02 | 1.39 to 77.04 |
| ALKP (U/L) | | | | |

| | | | | |
|---|---|---|---|---|
| ≤ 200 (Ref. category) | 1 | | | |
| >200 | 3.75 | 1.58 | 0.002 | 1.64 to 8.55 |

* Reference category: Aggressiveness Index (score=4)

Abbreviations: OR: Odds-Ratio; se(OR): Standard Error of Odds-Ratio; ESR: Erythrocyte Sedimentation Rate; CRP: C-Reactive Protein; ALKP: Alkaline Phosphatase; MTD: Maximum Tumor Diameter; AFP: Alpha-Fetoprotein; PVT: Portal Vein Thrombosis; AST: Aspartate Aminotransaminase; ALT: Alanine Aminotransferase; GGTP: Gamma Glutamyl Transpeptidase

Aggressiveness Index as sum of scores:

MTD (in terciles): MTD<4.5; 4.5 ≤ MTD ≤ 9.6; MTD>9.6; scores 1, 2, 3 respectively;

AFP (cut-off): AFP<100; 100 ≤ AFP ≤ 1000; AFP>1000 ng/ml; scores 1, 2, 3 respectively;

PVT (No/Yes): PVT (No); PVT (Yes); scores 1, 3 respectively;

Tumor Nodules (number): Nodules ≤ 3; Nodules>3; scores 1, 3 respectively.

**Table 4:** A: Logistic regression model of Aggressiveness Index score (4/>4)* on all parameters together in the model. B: Final Logistic regression model of Aggressiveness Index score (4/>4)* on all parameters in the backward stepwise method.

| Parameter | HR | Se(HR) | P | 95% C.I. |
|---|---|---|---|---|
| A) Total cohort | | | | |
| ESR (mm/hr) | | | | |
| ≤ 30 (Ref. category) | 1 | | | |
| >30 | 1.45 | 0.13 | <0.001 | 1.21 to 1.73 |
| CRP (mg/L) | | | | |
| ≤ 6 (Ref. category) | 1 | | | |
| >6 | 1.6 | 0.13 | <0.001 | 1.36 to 1.88 |
| ESR and CRP Combined | | | | |
| ESR ( ≤ 30) and CRP ( ≤ 6) (Ref. category) | 1 | | | |
| ESR (>30) and CRP (>6) | 2.53 | 0.38 | <0.001 | 1.88 to 3.41 |
| B) AFP<100 (IU/mL) | | | | |
| ESR (mm/hr) | | | | |
| ≤ 30 (Ref. category) | 1 | | | |
| >30 | 1.27 | 0.39 | 0.43 | 0.70 to 2.31 |
| CRP (mg/L) | | | | |
| ≤ 6 (Ref. category) | 1 | | | |
| >6 | 1.81 | 0.49 | 0.03 | 1.06 to 3.08 |
| ESR and CRP Combined | | | | |
| ESR ( ≤ 30) and CRP ( ≤ 6) (Ref. category) | 1 | | | |
| ESR (>30) and CRP (>6) | 3.43 | 2.33 | 0.05 | 0.91 to 12.98 |

Abbreviations: HR: Hazard-Ratio; se(HR): Standard Error of HR; ESR: Erythrocyte Sedimentation Rate (mm/hr); CRP: C-Reactive Protein (mg/L); AFP: Alpha-Fetoprotein.

**Table 5A:** Cox regression model on single parameters in the model.A, total cohort; B, patients with serum AFP<100 IU/mL.

The survival probability at 1, 2, 3 and 5 years (Table 5B) was then calculated for high and low values of ESR alone, CRP alone, and ESR and CRP together. Survival was significantly different, when high or low levels of ESR, CRP or the combination were compared, and the lowest survival at each time point was for high ESR and CRP combined.

| | ESR (mm/hr) | | | CRP (mg/L) | | |
|---|---|---|---|---|---|---|
| Variables * | ≤ 30 | >30 | p# | ≤ 6 | >6 | p# |
| Survival Probability at time (%) | | | | | | |
| 1 yr | 41.74 | 29.21 | 0.001 | 52.24 | 29.76 | <0.0001 |
| 2 yrs | 29.86 | 15.46 | <0.0001 | 35.53 | 17.62 | <0.0001 |
| 3 yrs | 18.55 | 11 | 0.008 | 22.82 | 10 | <0.0001 |
| 5 yrs | 10.72 | 6.87 | 0.09 | 12.24 | 5.48 | 0.0006 |

Abbreviations: ESR: Erythrocyte Sedimentation Rate; CRP: C-Reactive Protein. ¥Test z for proportions.

**Table 5B:** Comparisons of Survival Probability at single time, between categories of ESR, CRP, and ESR and CRP Combined.

## DISCUSSION

Rudolf Virchow first proposed that there might be an association between chronic inflammation and development of cancer in an 1863 book [20]. It is thought that up to a quarter of all human cancers are associated with inflammation related to bacterial and viral infections and this inflammation likely depends on the presence of active inflammatory cells in the tumor microenvironment [21-23]. Nuclear factor-kappaB (NF-kappaB) was shown to be a likely mechanistic link between inflammation and cancer, by controlling the resistance of tumor cells to apoptosis [24]. In HCC, chronic hepatitis B or C or alcoholism or metabolic syndrome/non alcoholicsteatohepatitis (NASH) cause chronic inflammation and subsequent HCC development, usually with multiple intermediate steps, including tissue remodeling and immune suppression in an altered tumor microenvironment [25,26]. Chronic activation of inflammatory signaling pathways results in the generation of reactive oxygen species and the inflammatory cells produce an array of growth factors, cytokines, chemokines, prostaglandins and pro-angiogenic factors. These factors include multiple interferons and interleukins such as IL-6. The acute phase reactants, particularly CRP and ESR have been studied in connection with inflammation and cancer for several decades [1,5 6]. IL-6 is an important NF-κB-regulated inflammatory mediator that enables tumor growth and inhibits apoptosis in a variety of human tumors and it reciprocally regulates CRP [27-29] as does STAT3 [30]. CRP, is synthesized locally by hepatocytes, HCC cells and several other cancer cell types [31-33]. It is not just a passive indicator of inflammation, but also has actions of its own, including modulation of N-cadherin [34] and growth control in some tumor models [35]. Erythrocyte sedimentation rate (ESR) is a systemic measure of inflammation, unlike CRP which is produced locally, and depends on altered blood viscosity in response to chronic inflammation (9-11), and is based on alterations in blood fibrinogen, globulins and other proteins. Patients with elevated ESR are considered to be at higher risk for cancer development, with a similar risk magnitude as CRP levels [36]. ESR is elevated in patients with many cancer types [10, 37-43], including HCC [44].

A great need in HCC is for sensitive and predictive markers of aggressive tumor behavior and patient survival. This need is reflected in the inability in the western world to detect most HCCs at a small and potentially curable size in most patients and because fewer than 50% of HCC patients have elevated levels of AFP [45,46], despite being the most useful blood-based HCC biomarker thus far. Recent studies have focused on indices of inflammation, including CRP, albumin and the ratios of lymphocytes, platelets and neutrophils [13,14,17,47-54], including in AFP negative HCC patients [55,56].

Initially, we separately considered patients with either high or low ESR or high or low CRP values (Table 1) and found significance for PVT and the Aggressiveness Index, for elevated ESR or CRP, and near significance for MTD. ESR and CRP were then combined and significance for the combination was found for high versus low combination ESR plus CRP for MTD, PVT and Aggressiveness Index. We next separately examined ESR and CRP subgroups for their related clinical and tumor characteristics in patients with either small (<5cm) or large tumors. For the CRP subgroups, there were no significant differences in patients with either small or large tumors. However, for ESR subgroups, patients with small tumors had significant differences in respect to blood AFP and their tumor Aggressiveness Index. For ESR subgroups in patients with large tumors, significant differences were seen in respect of all 4 tumor parameters under consideration (MTD, PVT, AFP and Aggressiveness Index). However, when ESR and CRP were combined (Table 3), significant subgroup differences were found also for patients with small tumors, in respect of PVT and Aggressiveness Index, as well as in respect of ALKP. This is a particularly useful finding, given the importance and difficulty in diagnosing patients with small and thus potentially curable HCCs.

A logistic regression model was obtained on the Aggressiveness Index on all parameters in the model. ORs were found to be significant for CRP or ALKP alone (Table 4). However, when ESR and CRP were combined, a significant OR of 11.66 was found for the combination compared to the reference, as well as in a final logistic regression model (OR 11.66). Lastly, the HRs in a Cox model were significant when ESR alone, CRP alone or ESR plus CRP were considered together (HR 2.53). It was especially encouraging to find that the combination was also significant for HCC patients who had low serum AFP values (HR 3.43).

In this report we show, for the first time we believe, a significant relationship between ESR values and indices of tumor aggressiveness, such as MTD and PVT, as well as a composite Aggressiveness Index. In addition, when the data was fitted in a Cox regression model on death, we found significant differences in the hazard ratios (HRs) for ESR and significant differences in survival between high and low ESR groups at 1, 2, 3, and 5 years of follow up. Cost is an ever-present consideration in medicine. A Google search showed costs for ESR in India from $1-2, in the USA from $14-40. CRP costs were found to be $5-15 in India and $12-50 in the USA. AFP costs were $5-12 in India and $40-70 in the USA. ESR thus also has the merit of relative cheapness in addition to its clinical value as shown here.

These findings suggest that ESR, a decadesold, ubiquitous and cheap lab test in all parts of the world, might have application in HCC management and prognosis, especially when combined with CRP. It is particularly attractive, being useful both in small size and in low AFP HCC patients.

## DISCLOSURE STATEMENT

The authors declare no conflict of interest. All authors have read and agree with this paper.

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**AUTHOR CONTRIBUTIONS AND ACKNOWLEDGEMENTS**

BIC and HA-concept and ideas.

BIC-writing; VG, RD-statistics; UK, KY, NE, AO, EA, HY, HS, AU, AB, SK, OU, YU, BG and AD-data collection, database formation and quality of data evaluation from original source documents.

**STATEMENT OF ETHICS**

This work complies with the guidelines of the World Medical Association, Declaration of Helsinki. This work was approved by each institution's IRB as documented in the methods section.

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