**Liver Cancer-Specific Serine Protease Inhibitor Kazal is a Potentially Novel Biomarker for the Early Detection of Hepatocellular Carcinoma**

Felix Lu1, Pir Ahmad Shah2, Abhishek Rao1, Cynthia Gifford-Hollingsworth3, Anne Chen1, Gary Trey2, Mina Soryal1, Arslan Talat2, Aysha Aslam2 Bilal Nasir2, Saad Choudhry2, Rizwan Ishtiaq2, Hanna Sanoff 4, Lanla F Conteh5, Anne Noonan5, Ke-Qin Hu6, Carl Schmidt7, Min Fu 8, Jesse Civan 9, Gary Xiao3, Daryl T.-Y. Lau2,Xuanyong Lu1\*

**Supplement 1. Sensitivity of LC-SPIK test Kit**

The sensitivity of LC-SPIK test kit was determined by detection of purified LC-SPIK purified from S2-3 cell line, which constantly expressed LC-SPIK20. A set of calibrators with different concentrations of LC-SPIK at 100ng/ml, 50ng/ml, 20ng/ml, 4ng/ml, 1ng/ml and 0ng/ml respectively was constructed as a standard curve. The limit of detection is 0.5ng/ml and the linear range for quantification was between 1ng/ml to 100ng/m (Suppl. 1). The R2 value of the linear regression equation ranged from 0.95-0.99 over 10 unique tests, showing excellent linearity of standard calibrator sets. Reliability was analyzed on more than 10 tests using IBM software SPSS 24 (IBM, Armonk, NY), and the Cronbach’s Alpha value for test was 0.993, suggesting excellent consistency.

*Suppl. 1: LC-SPIK standard curve and sensitivity.*

## Supplement 2. Statistical analysis of LC-SPIK in HCC patients, segmented by age, sex, race and LI-RADS score.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Cohort | Sub-group | # of Case | Mean of LC-SPIK (ng/ml) | P-value for comparisons between subset group\* |
| Age | <35\*\* | 1 | 73.0 | 0.353 |
| 36-50 | 14 | 42.0 |
| 51-60 | 58 | 39.4 |
| >60 | 91 | 48.7 |
| Sex | Female | 34 | 48.9 | 0.324 |
| Male | 130 | 44.0 |
| Race | White | 68 | 37.0 | 0.590 |
| Asian | 19 | 45.6 |
| Black | 71 | 53.0 |
| Other | 6 | 37.4 |
| LI-RADS | LR-4 | 43 | 42.9 | 0.420 |
| LR-5 | 110 | 47.4 |
| The mean difference is significant at the 0.05 level |
| \*The group sizes are unequal. Means for groups in homogeneous subsets are displayed. The harmonic mean of the group sizes is used.  |
| \*\* Only 1 case in Age < 35 group, it is excluded in analysis. |

**Supplement 3. Pan-SPIK did not interfere LC-SPIK test in blood**

Because LC-SPIK is a liver-cancer specific isoform of SPIK, which contains an additional fragment in its N-terminus, we evaluated if pancreatic SPIK (pan-SPIK) interferes with our LC-SPIK test. 3A.LC-SPIK was evaluated in 24 pancreatitis patients, who are expected to have elevated levels of pancreatic SPIK. We show that LC-SPIK levels in these patients (7.39ng/ml) were similar to those of healthy patients (7.38ng/ml, P > 0.99), and significantly lower than in HCC patients of our primary study (45.2ng/ml, P < 0.001). 3B.We confirm the elevated serum levels of pan-SPIK in our pancreatitis patients and observe that it does not interfere with our LC-SPIK kit.

*Suppl. 3. A. The Mean level of LC-SPIK in 164 HCC patients, 24 pancreatitis patients and 79 healthy people. B. The mean level of pan-SPIK in 24 pancreatitis patients and 79 healthy* people

**Supplement 4.** **Serum LC-SPIK levels in validation study**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Validation study | # of cases | Mean | 95% Confidence Interval for Mean | Minimum | Maximum |
| Lower Bound | Upper Bound |
| All HCC | 45 | 34.2 | 27.4 | 41.1 | 5 | 107 |
| Early HCC  | 30 | 26.6 | 21.2 | 32.0 | 5 | 64 |
| Cirrhosis | 57 | 19.3 | 16.9 | 21.6 | 3 | 45 |

**Supplement 5. Statistical analysis of serum LC-SPIK and AFP levels in Validation study**

|  |  |
| --- | --- |
|  |  |
| Validation Study (All HCC with cirrhosis control) | 95% Confidence Interval |
| Sample | Sensitivity | specificity | Cut-Off ng/ml | ROC Area | Lower Bound | Upper Bound |
| LC-SPIK | 80% | 72% | 21.5 | 0.74 | 0.64 | 0.85 |
| AFP | 51% | 81% | 20.0 | 0.73 | 0.64 | 0.83 |
|  |  |  |  |  |  |  |
| Validation Study (Early HCC with cirrhosis control) | 95% Confidence Interval |
| Sample | Sensitivity | specificity | Cut-Off ng/ml | ROC Area | Lower Bound | Upper Bound |
| LC-SPIK | 70% | 72% | 21.5 | 0.66 | 0.52 | 0.79 |
| AFP | 41% | 81% | 20.0 | 0.71 | 0.59 | 0.83 |

**Supplement 6. The distribution of LC-SPIK false-positive cases (LC-SPIK > 21.5ng/ml) in each control group.**

|  |  |  |
| --- | --- | --- |
| Cohorts | # of cases | LC-SPIK > 21.5ng/ml, Positive |
| Number | % |
| Total | 324 | 25 | 7.7% |
| **Cirrhosis** | 125 | **23** | **18.4%** |
| HCV | 37 | 2 | 5.4% |
| HBV | 83 | 0 | 0.0% |
| Healthy | 79 | 0 | 0.0% |