The Clinical Applications of Selected Circulating Tumor Biomarkers in Patients with Pancreatic Neuroendocrine Tumors/Neoplasms

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**Table S1**. The comparison of the tumour markers in patients with pancreatic neuroendocrine neoplasms (PNENs) and controls (Mann-Whitney U Test).

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| --- | --- | --- | --- |
| **Variable** | **PNEN patients**  **(n=115)** | **Controls**  **(n=40)** | **p** |
| median [interquartile range] | |  |
| Age (year) | 55.00 [42.00-65.00] | 48 [31-65] | NS |
| CY18 (U/L) | 64.67 [35.96-121.55] | 31.51 [23.05-45.67] | < 0.001 |
| Ferritin (ng/mL) | 75.43 [29.29-144.33] | 19.19 [8.80-94.81] | < 0.001 |
| CA19-9 (U/mL) | 9.13 [4.84-15.70] | 4.10 [2.19-7.66] | < 0.001 |
| CA125 (U/mL) | 5.88 [3.15-9.67] | 5.26 [3.37-7.95] | NS |
| AFP (µg/L) | 2.97 [1.95-4.76] | 2.64 [1.74-3.75] | NS |
| CEA (µg/L) | 1.13 [0.66-2.02] | 0.79 [0.49-1.51] | 0.03 |
| BMG (mg/L) | 1.20 [0.91-1.59] | 0.94 [0.84-1.12] | < 0.01 |

Abbreviations: PNEN, pancreatic neuroendocrine neoplasm; SD, standard deviation; BMI, body mass index; CY18, cytokeratin 18; CA125, cancer antigen 125; CA19-9, carbohydrate antigens 19-9; AFP, alpha-fetoprotein; CEA, carcinoembryonic antigen; BMG, beta-2 microglobulin; Ki-67, proliferation index; N/A, not applicable; NS, not significant; BMI, body mas index; CGA, chromogranin A; 5-HIAA, 5-hydroxyindoleacetic acid.

**Table S2.** The serum tumor markers assay metrics in the diagnosis of patients with pancreatic neuroendocrine neoplasm (PNEN).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **AUC**  **(95% CI)** | **SE** | **Z score** | **p** | **Youden**  **index J (%)** | **Cut-off value** | **Sensitivity**  **(%)** | **Specificity**  **(%)** | **Accuracy**  **(%)** |
| Age | 0.57 (0.45-0.69) | 0.06 | 1.17 | NS | 20.0 | 33.00 year | 92.20 | 27.50 | 75.50 |
| CY18 | 0.74 (0.65-0.83) | 0.05 | 5.10 | <0.0001 | 43 | 48.07 U/L | 65.20 | 77.50 | 68.40 |
| Ferritin | 0.70 (0.59-0.80) | 0.05 | 3.72 | 0.0002 | 41 | 23.75 ng/mL | 83.50 | 57.50 | 76.80 |
| CA19-9 | 0.72 (0.63-0.80) | 0.04 | 4.94 | <0.0001 | 36 | 4.28 U/mL | 78.30 | 57.50 | 72.90 |
| CA125 | 0.55 (0.45-0.64) | 0.05 | 0.93 | NS | 18 | 10.85 U/mL | 20.9 | 97.50 | 40.60 |
| AFP | 0.56 (0.46-0.66) | 0.05 | 1.10 | NS | 14 | 3.82 µg/L | 39.10 | 75.00 | 48.40 |
| CEA | 0.62 (0.52-0.71) | 0.05 | 2.36 | 0.0185 | 22 | 1.95 µg/L | 27.00 | 95.00 | 44.50 |
| BMG | 0.67 (0.58-0.75) | 0.04 | 3.74 | 0.0002 | 35 | 1.02 mg/L | 67.80 | 67.50 | 67.70 |

Abbreviations: AUC, area under the curve; CI, confidence interval; SE, standard error; and other abbreviations see Table S1

**Table S3.** The comparison of the tumour markers depending on the presence/absence of the metastatic disease in patients with pancreatic neuroendocrine neoplasms (Mann-Whitney U-Test).

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **Nonmetastatic disease**  **(n = 63)**  **Stage I, IIA, IIB, IIIA** | **Metastatic disease**  **(n = 52)**  **Stage IIIB, IV** | **p** |
| median [interquartile range] | |  |
| Age (year) | 53 ± 14 (53 [41-65]) | 53 ± 13 (58 [43-63] | NS |
| CY18 (U/L) | 76 ± 74 (51 [35-93] | 129 ± 114 (87 [47-183] | 0.01 |
| Ferritin (ng/mL) | 95 ± 90 (75 [27-128] | 117 ± 105 (81 [34-186] | NS |
| CA19-9 (U/mL) | 10 ± 8 (8 [4-14] | 16 ± 20 (11 [5-19] | NS |
| CA125 (U/mL) | 7 ± 9 (5 [3-8] | 17 ± 32 (7 [4-14] | 0.02 |
| AFP (µg/L) | 3 ± 2 (3 [2-5] | 5 ± 8 (3 [2-6] | NS |
| CEA (µg/L) | 1 ± 1 (1 [1-2] | 2 ± 2 (1 [1-3] | 0.01 |
| BMG (mg/L) | 1 ± (1 [1-2] | 7 ± 38 (1 [1-2] | NS |

Abbreviations: see Table S1.

**Table S4.** The serum tumor marker assay metrics in the differentiation of pancreatic neuroendocrine neoplasm (PNEN) depend on the presence/absence of metastatic disease.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **AUC**  **(95% CI)** | **SE** | **Z score** | **p** | **Youden**  **index J (%)** | **Cut-off value** | **Sensitivity**  **(%)** | **Specificity**  **(%)** | **Accuracy**  **(%)** |
| Age | 0.52 (0.41-0.63) | 0.05 | 0.37 | NS | 15 | 56.00 year | 57.70 | 57.10 | 57.40 |
| CY18 | 0.66 (0.54-0.75) | 0.05 | 2.79 | 0.0053 | 30 | 70.79 U/L | 61.50 | 68.30 | 65.20 |
| Ferritin | 0.56 (0.45-0.67) | 0.06 | 1.13 | NS | 23 | 141.99 ng/mL | 38.50 | 84.10 | 63.50 |
| CA19-9 | 0.57 (0.46-0.68) | 0.05 | 1.28 | NS | 17 | 15.70 U/mL | 34.60 | 82.50 | 60.90 |
| CA125 | 0.64 (0.53-0.74) | 0.05 | 2.56 | 0.0104 | 29 | 9.84 U/mL | 38.50 | 90.50 | 67.00 |
| AFP | 0.56 (0.46-0.67) | 0.05 | 1.16 | NS | 18 | 2.29 µg/L | 76.90 | 41.30 | 57.40 |
| CEA | 0.64 (0.55-0.74) | 0.05 | 2.74 | 0.0062 | 24 | 0.58 µg/L | 94.20 | 30.20 | 59.10 |
| BMG | 0.60 (0.50-0.70) | 0.05 | 1.88 | NS | 20 | 0.97 mg/L | 80.80 | 39.70 | 58.30 |

Abbreviations: see Table S3.

**Table S5.** Tumor markers in patients with pancreatic neuroendocrine neoplasm according to the extent of disease.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **TNM Stages** | | | **Kruskal-Wallis Test (KWT)**  **Chi-square test** (χ2**)** | | |
|  | **LD**  **(n= 63)** | **RM**  **(n= 8)** | **DM**  **(n= 44)** |  | | |
|  | **Median** | | | χ2 | KWT | p |
| Age (year) | 53.00 | 56.00 | 58.00 | 2.60 | 0.55 | NS |
| CY18 (U/L) | 51.07 | 72.05 | 98.36 | 9.53 | 9.97 | < 0.01 |
| Ferritin (ng/mL) | 75.43 | 42.81 | 90.15 | 0.60 | 3.30 | NS |
| CA19-9 (U/mL) | 8.05 | 14.67 | 10.86 | 1.63 | 2.25 | NS |
| CA125 (U/mL) | 5.16 | 7.28 | 7.24 | 3.64 | 6.28 | < 0.05 |
| AFP (µg/L) | 2.80 | 2.46 | 3.22 | 2.55 | 5.57 | NS |
| CEA (µg/L) | 0.91 | 0.91 | 1.45 | 6.55 | 11.20 | < 0.01 |
| BMG (mg/L) | 1.12 | 1.09 | 1.28 | 3.14 | 4.73 | NS |
| BMG (mg/L) | 1.12 | 1.09 | 1.28 | 3.14 | 4.73 | NS |

Abbreviations: see Table S1; LD, localized disease; RM, regional metastasis; DM, distant metastasis.

**Table S6.** Tumor markers in patients with pancreatic neuroendocrine neoplasm according to the TNM stages of the disease (I-IIIA, IIIB, IV).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **Stage**  **I, IIA, IIB, IIIA**  **N0M0** | **Stage**  **IIIB**  **N1M0** | **Stage**  **IV**  **N1M1** | | | **(chi-square test) (**χ2)  **Kruskal-Wallis Test (KWT)** | | |
|  | **n = 63** | **n = 8** | **LM**  **n = 12** | **LNM and LM**  **n = 22** | **OM**  **n = 10** |
|  | **Median** | | | | | χ2 | KWT | p |
| Age (year) | 53.00 | 56.00 | 57.50 | 56.00 | 59.00 | 3.37 | 2.12 | NS |
| CY18 (U/L) | 51.07 | 77.51 | 65.59 | 86.71 | 192.00 | 13.17 | 15.21 | < 0.05 |
| Ferritin (ng/mL) | 75.43 | 29.29 | 72.37 | 70.19 | 186.21 | 5.04 | 9.33 | NS |
| CA19-9 (U/mL) | 8.23 | 13.63 | 8.46 | 11.32 | 12.045 | 2.62 | 2.00 | NS |
| CA125 (U/mL) | 5.18 | 7.37 | 5.84 | 5.61 | 12.92 | 7.84 | 10.22 | < 0.05 |
| AFP (µg/L) | 2.80 | 2.80 | 3.59 | 3.3 | 3.055 | 1.61 | 3.78 | NS |
| CEA (µg/L) | 0.91 | 0.79 | 1.18 | 1.31 | 2.65 | 9.99 | 13.23 | 0.01 |
| BMG (mg/L) | 1.12 | 1.11 | 1.2 | 1.28 | 1.71 | 4.69 | 7.47 | NS |

Abbreviations: see Table S1; LNM, lymph node metastasis; LM, liver metastasis; OM, other metastasis.

**Table S7.** Tumor markers in patients with pancreatic neuroendocrine neoplasm according to TNM stages of the disease (I-II, III, IV).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **TNM Stages** | | | **Chi-square test** (χ2**)**  **Kruskal-Wallis Test (KWT)** | | |
|  | **I+II**  **(n= 57)** | **III**  **(n= 14)** | **IV**  **(n= 44)** |  | | |
|  | **Median** | | | χ2 | KWT | p |
| Age (year) | 53.00 | 56.00 | 58.00 | 2.52 | 0.43 | NS |
| CY18 (U/L) | 48.30 | 72.05 | 98.36 | 9.80 | 10.95 | < 0.01 |
| Ferritin (ng/mL) | 73.45 | 94.87 | 90.15 | 0.24 | 3.05 | NS |
| CA19-9 (U/mL) | 8.41 | 10.84 | 10.86 | 0.65 | 2.00 | NS |
| CA125 (U/mL) | 5.16 | 6.24 | 7.24 | 1.92 | 5.41 | NS |
| AFP (µg/L) | 2.63 | 2.89 | 3.22 | 1.29 | 4.13 | NS |
| CEA (µg/L) | 0.91 | 1.08 | 1.45 | 5.67 | 10.96 | < 0.01 |
| BMG (mg/L) | 1.19 | 1.06 | 1.28 | 3.47 | 4.78 | NS |

Abbreviations: see Table S1; TNM, tumour-nodulus-metastases…….

**Table S8.** Tumor markers in patients with pancreatic neuroendocrine neoplasm according to the grade of disease.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **NET G1**  **(n = 60)** | **NET G2**  **(n = 47)** | **NET G3 and NEC**  **(n = 8)** | **Kruskal-Wallis Test (KWT)**  **Chi-square test** (χ2**)** | | |
|  | **Median** | | | χ2 | KWT | p |
| Age (year) | 54.50 | 55.00 | 59.50 | 2.25 | 0.86 | NS |
| CY18 (U/L) | 57.48 | 69.87 | 182.98 | 6.35 | 9.59 | < 0.01 |
| Ferritin (ng/mL) | 77.38 | 67.02 | 110.83 | 1.09 | 0.92 | NS |
| CA19-9 (U/mL) | 9.16 | 8.75 | 12.60 | 0.68 | 0.43 | NS |
| CA125 (U/mL) | 5.99 | 5.61 | 12.54 | 2.52 | 7.36 | < 0.05 |
| AFP (µg/L) | 2.99 | 2.68 | 3.15 | 1.32 | 1.64 | NS |
| CEA (µg/L) | 0.93 | 1.23 | 1.82 | 2.09 | 5.46 | NS |
| BMG (mg/L) | 1.20 | 1.20 | 1.29 | 0.01 | 1.14 | NS |

Abbreviations: see Table S1; NET G1, neuroendocrine tumour grade 1; NET G2, neuroendocrine tumour grade 2; NET G3, neuroendocrine tumour grade 3; NEC, neuroendocrine carcinoma.

**Table S9.** Spearman’s coefficients of the relationships among analyzed variables in patients with pancreatic neuroendocrine neoplasm.

|  |  |  |
| --- | --- | --- |
| **Variables** | **R Spearman** | **p** |
| AFP & BMG | 0.21 | < 0.05 |
| age & BMG | 0.34 | < 0.01 |
| age & CEA | 0.29 | < 0.01 |
| age & CY18 | 0.20 | < 0.05 |
| age & Ferritin | 0.42 | < 0.01 |
| CA125 & BMG | 0.19 | < 0.05 |
| CA125 & CEA | 0.23 | < 0.05 |
| CA19-9 & CA125 | 0.32 | < 0.01 |
| CA19-9 & CEA | 0.19 | < 0.05 |
| CY18& AFP | 0.29 | < 0.01 |
| CY18 & BMG | 0.32 | < 0.01 |
| CY18 & CA125 | 0.33 | < 0.01 |
| CY18 & CEA | 0.20 | < 0.05 |
| CY18 & Ferritin | 0.33 | < 0.01 |
| Ferritin & BMG | 0.26 | < 0.01 |

Abbreviations: see Table S1.