

**Supplementary Table S1. Participant details and clinicopathological characteristics in biomarker discovery stage**

	ESCC (n=20)	Normal control (n=20)	p value
Age (Mean, range)	56, 45-68	56, 40-70	0.977 <sup>†</sup>
Gender			
Male	17	16	1.000 <sup>‡</sup>
Female	3	4	
T stage			
T1+T2	1		
T3+ T4	19		
N stage			
Negative	2		
Positive	18		
TNM stage			
III	4		
IV	16		

ESCC, oesophageal squamous cell carcinoma. <sup>†</sup>Mann-Whitney U test. <sup>‡</sup>Pearson chi-square test.

**Supplementary Table S2. Clinicopathological characteristics of all individuals by tumour type and subgroup in biomarker assessment stage**

	Normal control	Tumor type		
		ESCC	EJA	Stomach cancer
No. of participants	333	287	237	207
From CHSUMC	333	287	204	152
From SYSUCC	-	-	34	55
Age (years)	56 (40-79)	59 (41-83)	64 (31-87)	57 (26-81)
Gender				
Male	239	208	189	136
Female	94	79	48	71
T stage				
Tis+T1+T2		74	25	34
T3+ T4		213	212	173
N stage				
Negative		144	73	57
Positive		143	164	150
M stage				
M0		298	231	182
M1		0	6	25
TNM stage <sup>†</sup>				
0+I+II (early)		118	56	66
III+IV (advanced)		169	181	141

<sup>†</sup>All TNM stages in ESCC, EJA, and stomach cancer were pTNM stages. CHSUMC, Cancer Hospital of Shantou University Medical College; SYSUCC, Sun Yat-Sen University Cancer Centre. ESCC, oesophageal squamous cell carcinoma. EJA, oesophagogastric junction adenocarcinoma.

**Supplementary Table S3. Clinicopathological characteristics of participants evaluated in ESCC validation cohorts**

	Validation cohort 1 (CHSUMC)		Validation cohort 2 (SYSUCC)	
	ESCC (n=307)	Normal (n=165)	ESCC (n=262)	Normal (n=190)
Age (years)	60 (42-77)	57 (40-84)	61 (39-83)	56 (41-80)
Gender				
Male	232	110	208	147
Female	75	55	54	43
T stage				
Tis	6		7	
T1+T2	72		98	
T3+ T4	229		157	
N stage				
Negative	145		133	
Positive	162		129	
M stage				
M0	307		261	
M1	0		1	
pTNM stage				
0+I+II (early)	97		141	
III+IV (advanced)	210		121	

CHSUMC, Cancer Hospital of Shantou University Medical College; SYSUCC, Sun Yat-Sen University Cancer Centre.  
ESCC, oesophageal squamous cell carcinoma.

**Supplementary Table S4. 113 candidate Proteins/Cytokines displaying higher levels in patients with oesophageal squamous cell carcinoma than normal controls (fold change > 1.5) with the use of Human Antibody Array AAH-BLG-1000**

Protein/Cytokine name	Fold change	p value (Student t test)
GDF3	1.8035028	<0.001
Kallikrein 2	1.6731764	0.001
IGFBP-3	1.5227792	<0.001
IL-17R	1.5404811	0.0012
CRIM 1	2.4942821	0.0020
Hepassocin	1.6741045	0.0035
SAA	1.8737404	0.0035
IGFBP-2	1.5690116	0.0020
TGF-beta RII	1.7093058	0.0027
Tie-2	1.6903881	0.0031
Vasorin	1.5378916	0.0036
MMP-9	1.8050232	0.0058
G-CSF R/CD 114	1.5088181	0.0036
TNF RI/TNFRSF1A	1.6451376	0.0048
IL-1 F8/FIL1 eta	1.6211737	0.0069
uPAR	1.5849424	0.0072
IL-20 R alpha	1.5916308	0.0062
<b>IGFBP-1</b>	<b>1.7715375</b>	<b>0.0087</b>
D-Dimer	1.6075582	0.0109
Cytokeratin 8	1.5482369	0.0084
IL-1 sRI	1.5593007	0.0303
IL-2 R alpha	1.5464675	0.0132
FLRG	1.5356399	0.0114
TL1A/TNFSF15	1.6961292	0.0127
Tarc	1.530441	0.0135
MMP-13	1.5900909	0.0153
Cyclin D1	1.5619329	0.0145
TIMP-4	1.5036094	0.0155
VEGF-C	1.5676667	0.0110
Endoglin/CD105	1.5716644	0.0157
TWEAK/TNFSF12	1.6882888	0.0145
HCC-4/CCL16	1.5318565	0.0151
TRAIL R4/TNFRSF10D	1.6440075	0.0148
IL-2 R gamma	1.5366708	0.0162
Tie-1	1.5736473	0.0183
FGF R3	1.5044494	0.0174
TRAIL/TNFSF10	1.5620742	0.0168
MMP-15	1.6827519	0.0184
IL-31	1.5694644	0.0199
SSEA-1	1.5138386	0.0183
11b-HSD1	1.6855077	0.0234
TPA	1.634865	0.0214
Activin B	1.5602187	0.0204
NAIP	2.0679363	0.0223
Pentraxin3/TSG-14	1.6804978	0.0238
SCF R/CD117	1.7447789	0.0251

TRAIL R1/DR4/TNFRSF10A	1.629491	0.0265
E-Selectin	1.5091642	0.0269
CRTAM	1.7215272	0.0265
IL-6 R	1.5060397	0.0261
IL-31 RA	1.5151084	0.0221
TGF-beta RIIB	1.6557479	0.0263
IL-2 R beta/CD122	1.5955901	0.0285
FADD	1.6100827	0.0274
IL-10 R alpha	1.5291912	0.0293
Kremen-1	2.1217453	0.0312
IL-20	1.5969367	0.0310
BMP-9	1.606016	0.0295
PDGF-C	1.5640764	0.0245
Lipocalin-2	1.6206074	0.0356
Osteocalcin	1.664953	0.0307
Siglec-5/CD170	1.5354307	0.0291
S100A10	1.6715426	0.0301
FGF-BP	1.630238	0.0315
ADAM-9	1.6024065	0.0271
Activin RIA/ALK-2	1.6983134	0.0397
CV-2/Crossveinless-2	1.5351625	0.0346
Osteoprotegerin/TNFRSF11B	1.672582	0.0370
LRP-6	1.5854083	0.0354
WIF-1	1.5593254	0.0415
Persephin	1.6455155	0.0391
CD200	1.7097643	0.0391
Kallikrein 10	1.6372859	0.0407
IL-28A	1.5445768	0.0402
OX40 Ligand/TNFSF4	1.5543465	0.0395
TLR1	1.5361133	0.0434
Leptin (OB)	1.5429587	0.0338
Prohibitin	1.5397471	0.0328
ENA-78	1.5301049	0.0450
2B4	1.6332297	0.0440
IFN-alpha/beta R2	1.5173295	0.0403
FGF-21	1.5427336	0.0439
SLPI	1.5227405	0.0359
VEGI/TNFSF15	1.5167945	0.0483
Osteocrin	1.6212424	0.0466
LRP-1	1.5125739	0.0448
EDAR	1.5462306	0.0513
EMAP-II	1.5270894	0.0523
Insulin	1.6800395	0.0570
Soggy-1	1.5118312	0.0554
Flt-3 Ligand	1.5175439	0.0627
IL-22 BP	1.5020193	0.0582
Thymopoietin	1.6221846	0.0623
MMP-12	1.7369081	0.0629
KLF4	1.6683389	0.0627
Eotaxin/CCL11	1.5006744	0.0663

PPP2R5C	1.7309814	0.0754
Activin C	1.6981542	0.0907
Defensin	1.6560152	0.1271
ApoH	1.6123374	0.0974
Lyn	1.5705767	0.1151
FGF-12	1.5011377	0.0993
GPI	1.5404518	0.1275
PI 3Kinase p85 beta	1.8410221	0.1463
NPTX1	1.5102713	0.1431
hCGb	1.5772239	0.1338
IFN-gamma R1	1.8372062	0.1420
VIP Receptor 2	1.5293754	0.1310
SERPING1	1.8440742	0.1586
ACTH	1.5051708	0.1440
Layilin	1.9195336	0.1627
MMP-25/MT6-MMP	1.7710939	0.1475
Orexin B	2.1162364	0.1700

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**Supplementary Table S5. 39 candidate Proteins/Cytokines displaying higher levels in patients with oesophageal squamous cell carcinoma than normal controls (fold change > 1.5) with the use of with Human Cytokine Array AAH-CYT-G4000**

Protein/Cytokine name	Fold change	p value (Student t test)
MICA	1.87296537	<0.001
EGF	2.045899058	<0.001
OPN	3.205881542	<0.001
ErbB3	1.608572995	0.001
MMP-8	2.013110101	0.003
MIF	3.092384427	0.001
beta2 M	1.718732038	0.003
Ferritin	2.61888299	0.004
CEACAM-1	1.571837362	0.003
MIP-1beta	2.470839554	0.009
<b>IGFBP-1</b>	<b>1.56596948</b>	<b>0.011</b>
MMP-1	2.359251189	0.016
IL-8	2.990857414	0.022
uPAR	1.74913755	0.019
MCP-2	2.104978619	0.023
M-CSF	1.910152053	0.009
HGF	1.854534665	0.004
BLC	2.681199637	0.023
LGALS7	1.64183532	0.043
MMP-10	1.936992913	0.055
TNFSF14	3.977380107	0.062
HVEM	1.63389031	0.067
NRG1-beta1	1.649826306	0.060
IP-10	1.948766859	0.117
MIP-1alpha	8.279382918	0.120
MCP-3	4.409390824	0.123
GITR-Ligand	1.654607598	0.006
Furin	1.543881108	0.097
IL-1beta	6.093801511	0.147
TGF-alpha	1.771109789	0.017
FGF-7	3.980751443	0.155
TGF-beta 3	6.470047599	0.171
MIG	3.396202953	0.188
GDNF	3.451404578	0.162
IL-16	4.359289582	0.179
GCP-2	5.158204396	0.172
KIM1/TIM1	1.527796623	0.173
SCF	3.4696268	0.196
FGF-6	4.243327461	0.193

**Supplementary Table S6. Relationship between positive rates of IGFBP-1 and clinicopathologic features by tumour type in biomarker assessment stage**

	ESCC			EJA			Stomach cancer		
	n	positive (%)	p	n	positive (%)	p	n	positive (%)	p
<b>Patient age</b>									
≤55	100	65 (65.0%)	0.144	34	22 (64.7%)	0.060	82	42 (51.2%)	<0.001
>55	187	137 (73.3%)		203	161 (79.3%)		125	110 (88.0%)	
<b>Patient gender</b>									
Male	208	161 (77.4%)	<0.001	189	149 (78.8%)	.238	136	110 (80.9%)	0.001
Female	79	41 (51.9%)		48	34 (70.8%)		71	42 (59.2%)	
<b>T stage</b>									
Tis+T1+T2	74	46 (62.1%)	0.072	25	19 (76.0%)	0.878	34	18 (52.9%)	0.002
T3+ T4	213	156 (73.2%)		212	164 (77.4%)		173	136 (78.6%)	
<b>N stage</b>									
Positive	144	105 (72.9%)	0.346	164	125 (76.2%)	0.584	57	37 (64.9%)	0.087
Negative	143	97 (67.8%)		73	58 (79.5%)		150	115(76.7%)	
<b>Early-stage VS advanced-stage</b>									
Early (0+I+II)	118	84 (71.2%)	0.803	56	47 (83.9%)	0.171	66	41 (62.1%)	0.012
Advanced (III+IV)	169	118 (69.8%)		181	136 (75.1%)		141	111 (78.7%)	

NOTE: Statistical significance was determined by means of Chi-squared test. ESCC, oesophageal squamous cell carcinoma. EJA, oesophagogastric junction adenocarcinoma.



**Supplementary Table S7· Diagnostic performance of IGFBP-1 for ESCC in biomarker validation stage**

	AUC (95%CI)	Sensitivity (95%CI)	Specificity (95%CI)	PPV	NPV	PLR	NLR
<b>Validation cohort 1</b>							
ESCC (all stages) VS. Control	0·887 (0·858-0·916)	70·68% (65·25%-75·72%)	89·09% (83·31%-93·41%)	92·33%	62·07%	6·478	0·329
Early stage ESCC VS. Control	0·849 (0·799-0·899)	67·01% (56·73%-76·22%)	89·09% (83·31%-93·41%)	78·30%	82·14%	6·142	0·370
Advanced stage ESCC VS. Control	0·905 (0·875-0·934)	71·90% (65·31%-77·87%)	89·09% (83·31%-93·41%)	89·35%	71·36%	6·590	0·315
<b>Validation cohort 2</b>							
ESCC (all stages) VS. Control	0·909 (0·883-0·934)	73·28% (67·49%-78·54%)	87·89% (82·39%-92·17%)	89·31%	70·43%	6·051	0·304
Early stage ESCC VS. Control	0·911 (0·880-0·941)	71·63% (63·43%-78·90%)	87·89% (82·39%-92·17%)	81·45%	80·67%	5·915	0·323
Advanced stage ESCC VS. Control	0·906 (0·870-0·942)	75·21% (66·53%-82·60%)	87·89% (82·39%-92·17%)	79·81%	84·78%	6·211	0·282

CI, exact confidence interval; ESCC, oesophageal squamous cell carcinoma; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value.

**Supplementary Table S8. Relationship between positive rates of IGFBP-1 and clinicopathologic features of ESCC patients in biomarker validation stage**

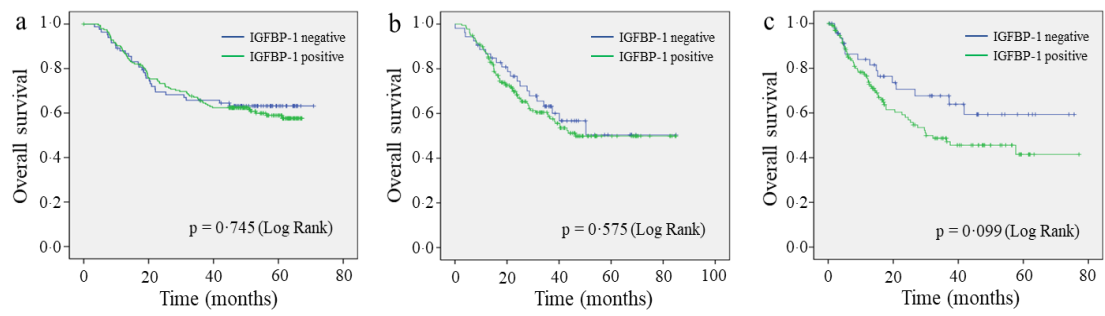
	Validation cohort 1 (CHSUMC)			Validation cohort 1 (CHSUMC)		
	n	Positive (%)	p	n	Positive (%)	p
<b>Patient age</b>						
≤55	76	48 (63.2%)	0.033	69	47 (68.1%)	0.161
>55	231	175 (75.8%)		193	148 (76.7%)	
<b>Patient gender</b>						
Male	232	177 (76.3%)	0.012	208	162 (77.9%)	0.012
Female	75	46 (61.3%)		54	33 (61.1%)	
<b>T stage</b>						
Tis + T1 + T2	78	51 (65.4%)	0.096	105	70 (66.7%)	0.019
T3 + T4	229	172 (75.1%)		157	125 (79.6%)	
<b>N stage</b>						
Positive	162	118 (72.8%)	0.933	129	95 (73.6%)	0.774
Negative	145	105 (72.4%)		133	100 (75.2%)	
<b>Early-stage VS advanced-stage</b>						
Early (0+I+II)	97	66 (68.0%)	0.219	141	102 (72.3%)	0.403
Advanced (III+IV)	210	157 (74.8%)		121	93 (76.9%)	

CHSUMC, Cancer Hospital of Shantou University Medical College; SYSUCC, Sun Yat-Sen University Cancer Centre; ESCC, oesophageal squamous cell carcinoma.

**Supplementary Table S9. Diagnostic performance of different biomarkers in ESCC**

	AUC (95%CI)	Sensitivity (95%CI)	Specificity (95%CI)	PPV	NPV	PLR	NLR
<b>All stages</b>							
CEA	0.542 (0.443-0.642)	14.58% (8.21%-23.26%)	93.33% (81.73%-98.60%)	82.35%	33.85%	2.186	0.915
Cyfra21-1	0.689 (0.603-0.775)	43.75% (33.64%-54.25%)	86.67% (73.21%-94.95%)	87.51%	41.92%	3.282	0.649
SCCA	0.581 (0.489-0.673)	32.29% (23.10%-42.61%)	93.33% (81.73%-98.60%)	91.18%	39.23%	4.481	0.725
CEA+ Cyfra21-1+SCCA	0.707 (0.623-0.791)	41.67% (31.68%-52.18%)	93.33% (81.73%-98.60%)	93.03%	42.84%	6.247	0.625
IGFBP-1	0.895 (0.845-0.948)	75.00% (65.12%-83.28%)	84.44% (70.54%-93.51%)	91.14%	61.27%	4.820	0.296
<b>Early stage</b>							
CEA	0.542 (0.427-0.656)	16.98% (8.07%-29.80%)	93.33% (81.73%-98.60%)	75.00%	48.82%	2.546	0.890
Cyfra21-1	0.673 (0.567-0.779)	39.62% (21.89%-48.36%)	86.67% (73.21%-94.95%)	77.79%	54.91%	2.972	0.697
SCCA	0.495 (0.376-0.615)	28.30% (16.79%-42.38%)	93.33% (81.73%-98.60%)	83.34%	52.48%	4.243	0.768
CEA+ Cyfra21-1+SCCA	0.692 (0.587-0.797)	39.62% (26.45%-54.00%)	93.33% (81.73%-98.60%)	87.50%	56.74%	5.940	0.647
IGFBP-1	0.901 (0.843-0.960)	77.36% (63.79%-87.72%)	84.44% (70.54%-93.51%)	85.42%	75.99%	4.972	0.268
<b>Advanced stage</b>							
CEA	0.543 (0.422-0.665)	11.63% (3.885%-25.08%)	93.33% (81.73%-98.60%)	62.53%	52.46%	1.744	0.947
Cyfra21-1	0.709 (0.596-0.821)	48.84% (33.31%-64.54%)	86.67% (73.21%-94.95%)	77.81%	63.90%	3.664	0.590
SCCA	0.687 (0.572-0.801)	37.21% (22.98%-53.28%)	93.33% (81.73%-98.60%)	84.22%	60.83%	5.579	0.673
CEA+ Cyfra21-1+SCCA	0.726 (0.619-0.834)	41.86% (27.01%-57.87%)	93.33% (81.73%-98.60%)	85.73%	62.65%	6.276	0.623
IGFBP-1	0.887 (0.817-0.957)	74.42% (58.83%-86.48%)	84.44% (70.54%-93.51%)	82.52%	79.14%	4.932	0.275

CEA, carcinoembryonic antigen; SCCA, squamous cell carcinoma antigen; CI, exact confidence interval; NC, normal controls; ESCC, oesophageal squamous cell carcinoma; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value.



**Supplementary Figure S1.** Kaplan-Meier analysis of the overall survival for serum IGFBP-1 in oesophageal squamous cell carcinoma (a), oesophagogastric junction adenocarcinomas (b) and stomach cancer (c) in biomarker assessment stage.