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Establishment and validation of reference intervals for tumor markers (AFP, CEA, CA19-9, CA15-3, CA125, PSA, HE4, Cyfra 21-1, and ProGRP) in primary care centers in Korea: A cross-sectional retrospective study

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Abstract

Background and Aims: The reference interval (RI) for a tumor marker may vary between populations, detection systems, and the methods used to obtain their values. The aims of this study were to establish age- and sex-specific RIs for the following nine common tumor markers and to validate the established RIs in Korean adults: alpha fetoprotein (AFP), carcinoembryonic antigen (CEA), cancer antigen (CA) 19-9, CA15-3, CA125, Human epididymis protein 4 (HE4), total prostate specific antigen, cytokeratin fragment (Cyfra) 21-1, and progastrin-releasing peptide (ProGRP).

Methods: This cross-sectional study consecutively selected 214,159 individuals (aged 18–98 years) who underwent health checkups at 16 health-promotion centers in 13 Korean cities. Finally, 62,752 examinees were used to establish the RIs after removing outliers. RIs were established using an indirect method according to the CLSI EP28-A3C guideline. The established RIs were validated by calculating the proportion of individuals outside each RI.

Results: Sex-related differences were observed for AFP, CEA, CA19-9, Cyfra 21-1, and ProGRP ($p < 0.05$): AFP, CEA and Cyfra 21-1 were higher in males, and CA19-9 and proGRP were higher in females. Most of the tumor markers except CA15-3 and CA125 increased with age: CA125 decreased at ≥50 years of age ($p < 0.05$), while CA15-3 did not vary with age. Less than 5% of subjects were outside all RIs (the 2.5th and 97.5th percentiles) established in the present study. Meanwhile, less than 3% of the healthy reference subjects fell outside the current and manufacturers' RIs of all tumor markers except Cyfra 21-1.

Conclusion: This study has determined age- and sex-specific RIs for nine common tumor markers in the healthy Korean population, which could be useful for clinicians making clinical decisions and assessments.

KEYWORDS

age-specific reference interval, reference interval, tumor marker, validation

1 | INTRODUCTION

Tumorigenesis does not happen in isolation, but rather in the complex milieu of structured tissues and organs. Chemical and physical forces exerted by microenvironment surrounding a tumor make development and progression of tumor.[1] Cancer could be also conceptualized as a multidimensional spatiotemporal "unity of ecology and evolution" pathological ecosystem.[2] Cancer is a major public health concern in Korea due to it being the leading cause of death.[3] Early diagnosis allows more effective therapeutic interventions for patients and hence reduced mortality.[4] There is no reported tumor marker provides a survival benefit in screening the general population. Nevertheless, tumor markers in general can play an important role as noninvasive tools in screening disease, predicting therapeutic efficacy, and in surveillance following surgical tumor excision in selected groups of patients.[5–7] Clinical application of tumor markers in routine clinical practice can be summarized: alpha fetoprotein (AFP) for hepatoma; carcinoembryonic antigen (CEA), and cancer antigen (CA) 19-9 for malignancy of colorectal, stomach, and pancreas; CA15-3 for breast cancer; CA125, and human epididymis protein 4 (HE4) for ovarian cancer; total prostate specific antigen (PSA) for prostate cancer; cytokeratin fragment (Cyfra) 21-1, and progastrin-releasing peptide (ProGRP) for lung cancer.

The measured level of a tumor marker in an individual is interpreted by comparing it with its corresponding reference interval (RI).[8] There are significant interregional and interlaboratory variations in RIs, which are attributable to several factors including variations in the characteristics of reference populations, the methods used to obtain RIs, and the analytic instruments used to determine tumor markers.[9,10] This situation means that laboratories should need to perform their own RI investigations to establish RIs specific for methods they apply and the populations they apply them to. Our institute established its own RIs for the following tumor markers 20 years ago: AFP, CEA, CA19-9, CA15-3, CA125 and PSA.[11] During the intervening period, the analytical method for the tumor markers changed from enzyme-linked immunosorbent assay (ELISA) to an electrochemiluminescence immunoassay (ECLIA), which makes it necessary to re-establish the RIs in the Korean population. Although a few studies have established RIs for tumor makers in Koreans,[12–14] those studies had some important limitations, such as small numbers of study subjects, restricted types of tumor markers, and using invasive sampling method such as ascites.

This study therefore aimed to establish age- and sex-specific RIs for the following nine common tumor markers and to validate the established RIs in the healthy Korean population: AFP, CEA, CA19-9, CA15-3, CA125, PSA, HE4, Cyfra 21-1, and ProGRP.

2 | MATERIALS AND METHODS

2.1 | Study subjects

This cross-sectional retrospective study consecutively selected 214,159 subjects who underwent health checkups at 16 health-promotion centers in 13 Korean cities between January 2019 and September 2021. The cases were collected in a central database and retrieved as required to calculate RIs of tumor markers. The self-reported personal medical history, subjective symptoms and signs, and lifestyle information were obtained from all participants during health checkups. Medical records related to cancers such as hepatoma, cancers of colorectal, stomach, pancreas, breast cancer, ovarian cancer, prostate cancer, and lung cancers, and other diseases were also reviewed. The following exclusion criteria were applied to ensure that unhealthy individuals were not included in the analysis: pregnant or lactating females, consuming three or more alcoholic drinks per day, hypertension, diabetes mellitus, dyslipidemia, renal dysfunction, obesity (body mass index; >30 kg/m^2), or positivity for HBsAg, anti-HCV, anti-HIV, or certain types of cancer.

2.2 | Laboratory analysis

Serum samples were obtained from health examinees in 16 health promotion centers and transported to a central core laboratory (MEDIcheck LAB), where tumor markers were measured using ECLIA on the Roche Cobas E801 system (Roche Diagnostics). The PreciControl Tumor Marker was used for quality control (QC). Three levels of QC were run individually at least once every 24 h. Westgard rules were used to evaluate internal QC. External QC was evaluated using the National External Quality Assurance Scheme conducted by the Korean Association of Quality Assurance for Clinical Laboratories. The precision and accuracy of the nine tumor markers were evaluated according to the EP15-A3.[15]

2.3 | Statistical analysis and calculation of RIs

We analyzed the pooled data of health examinees obtained from 16 health promotion centers to establish age- and sex-specific RIs for the following nine common tumor markers: AFP, CEA, CA19-9, CA15-3, CA125, PSA, HE4, Cyfra 21-1, and ProGRP. The RIs for these tumor markers were established using an indirect method. Tumor markers data were analyzed according to guideline EP28-A3C of the Clinical Laboratory Standardization Institute (CLSI).[16] Scatter and distribution plots were generated and used to inspect the data (Figure 1). The normality of the data was analyzed using the Skewness–Kurtosis test. Non-normally distributed data were transformed using the Box-Cox transformation. Outliers were removed using the Tukey test. The Harris and Boyd method was then used to decide whether partitioning the RI could be justified statistically. A nonparametric method was used to determine the RIs for all of the partitions.

Box plots were generated to display the variations in tumor markers according to age and sex. Multiple comparisons between age and sex groups were performed using the Wilcoxon rank sum test with pairwise comparison and the Bonferroni correction to compensate for alpha statistical errors. The Wilcoxon rank sum test and Kruskal–Wallis test were performed to compare parameters according to sex and age groups, respectively. Statistical analyses

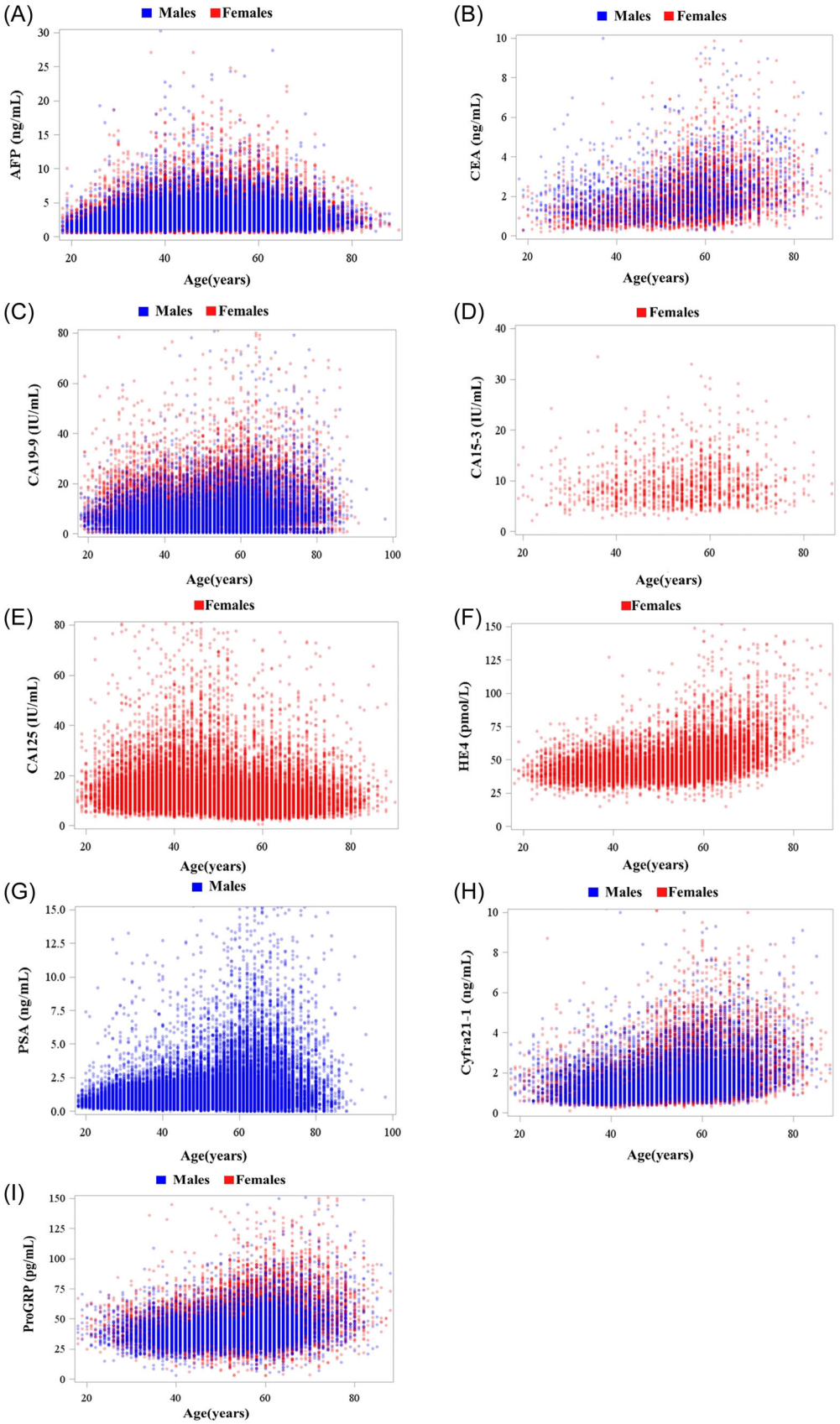

FIGURE 1 Scatter plot distributions of tumor markers: (A) alpha-fetoprotein (AFP), (B) carcinoembryonic antigen (CEA), (C) cancer antigen19-9 (CA19-9), (D) cancer antigen 15-3 (CA15-3), (E) cancer antigen125 (CA125), (F) human epididymis protein 4 (HE4), (G) prostate-specific antigen (PSA), (H) cytokeratin fragment 21-1 (Cyfra 21-1), and (I) Progastrin-releasing peptide (ProGRP).

were performed with SAS version 9.4 (SAS Institute Incorporated). All tests were 2-sided and $p < 0.05$ was considered indicative of statistical significance.

2.4 | Validation of RIs

We also validated the established RIs in the healthy Korean population. According to the tumor markers, the maximum number of 18,269 cases of apparently healthy people who underwent health checkups at 16 health promotion centers from October to December 2021 were obtained. The proportion of individuals outside an established RI was calculated to validate that RI, with the established RI accepted if this proportion was less than 5%.[16,17] We compared the proportions of subjects outside the RIs established in the present study with those of the current RIs and RIs from manufacturers.

TABLE 1 Number of reference subjects and excluded subjects from eligible subjects.

Tumor marker	Eligible subjects	Excluded subjects		Reference subjects	Reference subjects after removing outlier
		Total	Excluded conditions		
AFP	214,159	150,779	Malignant (122), benign (150,657)	63,380	62,752
CEA	17,649	9885	Malignant (11), benign (9874)	7764	7671
CA19-9	159,408	122,844	Malignant (326), benign (122,552)	36,534	34,896
CA15-3	2114	658	Malignant (24), benign (634)	1456	1450
CA125	84,569	35,706	Malignant (117), benign (35,589)	48,863	48,078
HE4	28,920	13,412	Malignant (57), benign (13,355)	15,508	15,182
PSA	89,281	7	Malignant (7)	89,274	86,477
Cyfra 21-1	63,319	32,027	Malignant (156), benign (32,822)	31,292	31,002
ProGRP	63,235	32,393	Malignant (155), benign (32,238)	30,842	30,195

Abbreviations: AFP, alpha-fetoprotein; CA19-9, cancer antigen19-9; CA15-3, cancer antigen 15-3; CA125, cancer antigen125; CEA, carcinoembryonic antigen; Cyfra 21-1, cytokeratin 19 fragment; HE4, human epididymis protein 4; ProGRP, progastrin releasing peptide; PSA, prostate specific antigen.

TABLE 2 Age- and sex-specific reference interval for tumor markers.

Tumor makers	Partitioning		Total	Established RIs			
	Age	Sex		95th percentiles	(95% CI)	97.5th percentiles	(95% CI)
AFP (ng/mL)	<30		3830	4.44	(4.32–4.61)	5.37	(5.11–5.59)
	30–39	M	2967	6.16	(5.92–6.39)	7.36	(7.00–7.88)
		F	5768	5.75	(5.59–5.92)	6.83	(6.53–7.22)
	40–49	M	4869	6.77	(6.61–6.96)	7.77	(7.58–8.07)
		F	9352	6.55	(6.37–6.73)	7.75	(7.57–8.01)
	50–59		17,890	6.64	(6.55–6.74)	7.71	(7.60–7.86)
	60–69		14,892	6.18	(6.07–6.29)	7.25	(7.10–7.43)
	≥70		3184	5.43	(5.24–5.83)	6.44	(6.15–6.85)
CEA (ng/mL)	<40	F	494	2.73	(2.45–3.07)	3.24	(2.85–3.83)
	40–49	F	793	3.02	(2.77–3.30)	3.49	(3.29–4.29)
	50–59	F	1846	3.78	(3.55–3.95)	4.38	(4.16–4.71)
	60–69	F	1772	4.32	(4.10–4.55)	5.02	(4.82–5.53)
	≥70	F	594	4.72	(4.65–4.89)	5.36	(5.21–5.65)
	All	M	2172				

TABLE 2 (Continued)

Tumor makers	Partitioning		Total	Established RIs			
	Age	Sex		95th percentiles	(95% CI)	97.5th percentiles	(95% CI)
CA19-9 (IU/mL)	<70	M	12,359	20.2	(19.74–20.69)	23.7	(23.20–24.30)
	≥70	M	1,719	24.8	(24.41–25.14)	28.78	(28.33–29.18)
	All	F	20,818				
CA15-3 (IU/mL)	All	F	1450	18.4	(17.40–19.63)	20.74	(20.03–22.13)
CA125 (IU/mL)	<50	F	14,474	29	(28.52–29.53)	34.13	(33.30–35.08)
	50–59	F	15,273	19.99	(19.60–20.40)	24.39	(23.71–25.20)
	60–69	F	14,647	18.68	(18.44–19.10)	22.2	(21.72–22.90)
	70–79	F	3418	21.1	(20.30–22.06)	26.13	(24.31–28.16)
	≥80	F	266	26.04	(22.44–33.18)	33.18	(27.35–47.18)
HE4 (pmol/L)	<40	F	2091	59.06	(57.90–60.80)	63.4	(61.90–65.30)
	40–49	F	2752	61.3	(60.32–62.90)	67.07	(65.06–69.10)
	50–59	F	4789	65.5	(64.60–66.90)	71.3	(70.10–73.30)
	60–69	F	4,603	73.3	(71.90–74.60)	81.2	(79.30–83.50)
	70–79	F	903	87.7	(85.90–90.40)	92.97	(90.20–95.70)
	≥80	F	44	89.5	(87.78–92.20)	90.7	(89.50–92.20)
PSA (ng/mL)	<50	M	34,149	1.82	(1.87–2.22)	2.22	(2.18–2.26)
	50–59	M	24,587	2.21	(2.28–2.70)	2.7	(2.65–2.76)
	60–69	M	22,381	2.77	(2.84–3.23)	3.23	(3.19–3.27)
	≥70	M	5360	3.08	(3.22–3.47)	3.47	(3.41–3.54)
Cyfra 211 (ng/mL)	<30		493	3.1	(2.90–3.40)	3.5	(3.30–4.20)
	30-39		2449	2.7	(2.60–2.80)	3.2	(3.00–3.50)
	40–49	M	1830	3.1	(3.00–3.30)	3.8	(3.50–4.00)
		F	3279	2.7	(2.60–2.80)	3.2	(3.10–3.30)
	50–59	M	2572	3.6	(3.40–3.70)	4.1	(4.00–4.40)
		F	7909	3.4	(3.30–3.50)	4.1	(4.00–4.20)
	60–69	M	2621	4.1	(4.00–4.30)	4.6	(4.50–5.00)
		F	7458	3.8	(3.70–3.80)	4.4	(4.30–4.50)
	≥70		2391	4.6	(4.50–4.80)	5.2	(5.00–5.40)
ProGRP (pg/mL)	<30		480	62	(60.80–65.50)	66.4	(63.80–70.30)
	30–49	M	3015	59.05	(57.90–60.16)	64.74	(62.99–66.90)
		F	4408	62.69	(61.50–63.40)	68.3	(66.90–70.66)
	50–59	M	2497	66.11	(64.60–67.49)	72.72	(70.70–74.60)
		F	7714	70.19	(69.20–71.20)	76.7	(75.60–78.10)
	60–69	M	2554	71.2	(69.54–73.52)	79.7	(76.20–82.10)
		F	7268	74.7	(73.90–75.50)	81.3	(79.30–82.60)
	70–79	M	666	79.45	(75.30–84.17)	85.05	(82.80–88.50)
		F	1427	83	(80.03–84.96)	86.88	(85.52–90.00)
	≥80		166	87.7	(82.90–94.66)	93.6	(87.70–95.50)

Abbreviations: AFP, alpha-fetoprotein; CA19-9, cancer antigen19-9; CA15-3, cancer antigen 15-3; CA125, cancer antigen125; CEA, carcinoembryonic antigen; CI, confidence interval; Cyfra 21-1, cytokeratin 19 fragment; F, females; HE4, human epididymis protein 4; M, males; ProGRP, progastrin releasing peptide; PSA, prostate specific antigen; RI, reference interval.

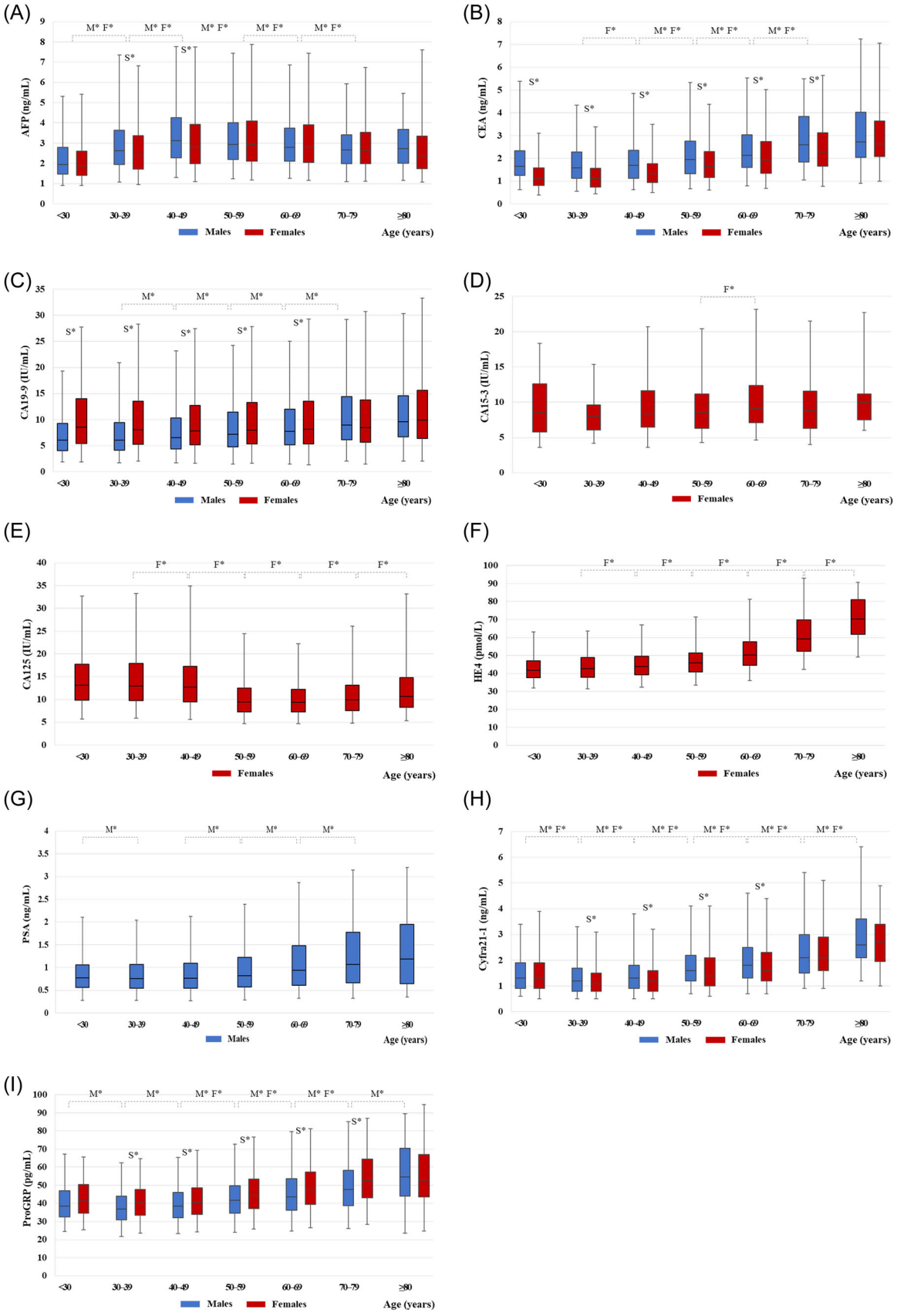

FIGURE 2 (See caption on next page)

3 | RESULTS

3.1 | Subjects for establishing RIs

This study initially enrolled 214,159 health examinees. To obtain healthy reference subjects, malignant and benign conditions such as inflammation, and benign tumors were excluded using sonography, endoscopy, and radiographic and laboratory tests. This yielded 62,752 examinees who were finally used to establish the RIs after removing outliers (Table 1).

3.2 | Establishment of RIs

Age- and sex-specific RIs for tumor markers are listed in Table 2, which groups data of the tumor markers by age and sex. The tumor markers required several age partitions except for CEA in males and CA19-9 in females. Sex partitions were required for all tested tumor markers. The 95th and 97.5th percentiles of the RIs for tumor markers with their 95% confidence intervals were established in each age and sex partitions using a nonparametric method.

3.3 | Age- and sex-related findings

Tumor markers varied with both age and sex (Figure 2). Sex-related differences were observed for AFP, CEA, CA19-9, Cyfra 21-1, and proGRP ($p < 0.05$): AFP, CEA, and Cyfra 21-1 were higher in males, and CA19-9 and ProGRP were higher in females. Most of the tumor markers except CA15-3 and CA125 increased with age: CA125 decreased at ≥50 years of age ($p < 0.05$), while CA15-3 did not vary with age.

3.4 | Validation and comparison of established RIs

Table 3 presents the results of validating the RIs established in this study and comparing them with current RIs and manufacturers' RIs. The validation of RIs was performed with 18,269 examinees. Less than 5% of subjects were outside the 97.5th percentiles of all RIs established in the present study. However, more than 5% of subjects were outside the 95th percentiles of RIs for several age and sex subgroups, such as across all age subgroups for PSA.

4 | DISCUSSION

We established nine common tumor markers for healthy Korean subjects based on medical records including the results of sonography, endoscopy, and radiographic and laboratory tests. Changes in tumor markers were also evaluated throughout adulthood according to age and sex. In addition, the RIs established in this study were validated and compared them with current RIs and manufacturers' RIs.

The CLSI recommends using a direct method with a healthy reference population to establish RIs,[16] but most laboratories have difficulties recruiting large numbers of reference subjects. The indirect method involves establishing RIs using a laboratory database in a laboratory information system. However, the indirect method could inevitably include unhealthy subjects, and so unhealthy subjects as identified based on the results of sonography, endoscopy, and radiographic and laboratory tests were excluded from the reference population used for establishing RIs in this study. Compared with the RIs currently used in our laboratory, which were established 20 years ago,[11] the upper RIs established in the present study were lower, which might be attributable to the exclusion of unhealthy subjects in the present study.

Updated RIs in adulthood were obtained in a large healthy population, and changes in tumor markers according to age and sex were identified in this study. Most of the tumor markers except CA15-3 and CA125 increased with age. Some previous studies[18–21] also showed that AFP, CEA, and CA19-9 increased with age. Yang et al.[20] reported that AFP, CEA, CA19-9, CA125, and Cyfra 21-1 differed significantly among age groups, which is consistent with our results. In contrast, Bjerner et al.[21] reported that CA125 and CA19-9 were independent of age over certain age ranges. They reported that the upper reference limits of CA125 and CA19-9 were 35.8 and 28.3 kU/L, respectively, in all adult age groups. Additionally, our results showed that the RIs of HE4 increased with age, whereas those of CA125 decreased at ≥50 years of age. The lower levels of CA125 at ≥50 years of age might be caused by the postmenopausal status, but the menopausal status could not be evaluated in the present study. A few studies[22,23] found that CA125 levels were significantly lower in postmenopausal females than in premenopausal individuals. In addition, sex-related differences were observed in AFP, CEA, CA19-9, Cyfra 21-1, and ProGRP in the present study: AFP, CEA, and Cyfra 21-1 were higher in males, and CA19-9 and ProGRP were higher in females. These sex-related differences in AFP, CEA and CA19-9 have also been reported previously.[19,20]

The RIs established in the present study were validated and compared with current RIs and manufacturers' RIs in our health

FIGURE 2 Box plots of tumor markers according to sex and age: (A) AFP (B) CEA (C) CA19-9 (D) CA15-3 (E) CA125 (F) HE4 (G) PSA (H) Cyfra 21-1 (I) ProGRP. Box limits and horizontal lines within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively. The difference in median values between sexes in each age group was determined using the Wilcoxon rank sum test. In each sex, comparison of median values among age groups was performed by Kruskal–Wallis test, and pairwise comparisons between adjacent age groups was performed using Wilcoxon rank sum test. *Bonferroni corrected $p < 0.05$.

TABLE 3 Validation of established RIs, and comparisons with current RIs and manufacturers' RIs.

	The established RIs[a]							The current RIs[b]					The manufacturer's RIs[c]				
	Partitioning			95 percentile		97.5 percentile		Subgroups		RI			Subgroups		RI		
Tumor makers	Age	Sex	Number of validated subjects	*n*	(%)	*n*	(%)	Age/Sex	Number of validated subjects	*n*	(%)	Upper reference limits	Age/Sex	Number of validated subjects	*n*	(%)	Upper reference limits
AFP	<30		616	39	(**6.3**)	15	(2.4)	All	18,269	233	(1.3)	8.9	All	18,269	715	(3.9)	7.0
	30–39	M	1093	50	(4.6)	27	(2.5)										
		F	1035	53	(**5.1**)	23	(2.2)										
	40–49	M	2,374	142	(**6.0**)	83	(3.5)										
		F	2301	148	(**6.4**)	78	(3.4)										
	50–59		5616	283	(**5.0**)	149	(2.7)										
	60–69		4364	250	(**5.7**)	131	(3.0)										
	≥70		870	59	(**6.8**)	31	(3.6)										
CEA	<40	F	70	0	-	0		All F	881	9	(1.0)	6.0	All	1,784	54	(3.0)	5.2
	40–49	F	152	4	(2.6)	2	(1.3)	All M	903	1	(0.1)	8.2					
	50–59	F	313	17	(**5.4**)	8	(2.6)										
	60–69	F	296	11	(3.7)	6	(2.0)										
	≥70	F	50	62	(**6.5**)	40	(4.2)										
	All	M	903														
CA19-9	<70	M	8,907	430	(4.8)	234	(2.6)	All	19,702	161	(0.8)	35.2	All	19,702	186	(0.9)	34
	≥0	M	451	506	(4.7)	285	(2.6)										
	All	F	10,344														
CA15-3	All	F	200	17	(**8.5**)	5	(2.5)	All F	200	0	-	25.0	All F	200	0	-	28.5
CA125	<50	F	2845	219	(**7.7**)	129	(4.5)	All F	7580	123	(1.6)	38.3	All F	7580	157	(2.1)	35
	50–59	F	2529	159	(**6.3**)	88	(3.5)										
	60–69	F	1884	94	(**5.0**)	46	(2.4)										
	70–79	F	293	21	(**7.2**)	8	(2.7)										
	≥80	F	29	1	(3.5)	1	(3.5)										
HE4	< 40	F	573	19	(3.3)	13	(2.3)	<30	105	1	(1.0)	59.93	<40	573	15	(2.6)	60.5
	40–49	F	1264	62	(4.9)	26	(2.1)	30–49	1732	51	(2.9)	64.02	40–49	1264	11	(0.9)	76.2
	50–59	F	1767	71	(4.0)	43	(2.4)	50–59	1767	41	(2.3)	72.06	50–59	1767	31	(1.8)	74.3
	60–69	F	1367	49	(3.6)	17	(1.2)	≥60	1586	0	-	93.84	60–69	1367	12	(0.9)	82.9
	70–79	F	209	4	(1.9)	0	-						≥70	219	0	-	104
	≥80	F	10	1	(**10.0**)	0	-										

TABLE 3 (Continued)

Tumor makers	The established RIs[a]							The current RIs[b]					The manufacturer's RIs[c]				
	Partitioning			95 percentile		97.5 percentile		Subgroups		RI			Subgroups		RI		
	Age	Sex	Number of validated subjects	*n*	(%)	*n*	(%)	Age/Sex	Number of validated subjects	*n*	(%)	Upper reference limits	Age/Sex	Number of validated subjects	*n*	(%)	Upper reference limits
PSA	<50	M	3412	175	(**5.1**)	96	(2.8)	All M	7898	44	(0.6)	3.8	<40	1280	147	(11.5)	1.4
	50–59	M	2344	140	(**6.0**)	72	(3.1)						40–49	2132	83	(3.9)	2
	60–69	M	1786	106	(**5.9**)	57	(3.2)						50–59	2344	42	(1.8)	3.1
	≥70	M	356	23	(**6.5**)	15	(4.2)						60–69	1786	5	(0.3)	4.1
													≥70	356	0	-	4.4
Cyfra 21-1	<30		145	3	(2.1)	1	(0.7)	All	10,987	635	(5.8)	3.3	All	10,987	635	(5.8)	3.3
	30–39		868	29	(3.3)	12	(1.4)										
	40–49	M	1690	57	(3.4)	28	(1.7)										
		F	1062	58	(**5.5**)	24	(2.3)										
	50–59	M	2045	83	(4.1)	51	(2.5)										
		F	1651	73	(4.4)	27	(1.6)										
	60–69	M	1522	66	(4.3)	41	(2.7)										
		F	1400	66	(4.7)	35	(2.5)										
	Over 70		604	41	(**6.8**)	26	(4.3)										
ProGRP	<30		143	3	(2.1)	3	(2.1)	All	10,771	160	(1.5)	77.7	All	10,771	160	(1.5)	77.7
	30–49	M	2203	110	(**5.0**)	50	(2.3)										
		F	1376	63	(4.6)	32	(2.3)										
	50–59	M	2006	92	(4.6)	50	(2.5)										
		F	1630	59	(3.6)	25	(1.5)										
	60–69	M	1486	77	(**5.2**)	25	(1.7)										
		F	1356	64	(4.7)	25	(1.8)										
	70–79	M	276	12	(4.4)	4	(1.5)										
		F	248	4	(1.6)	2	(0.8)										
	≥80		47	1	(2.1)	0	-										

Note: *n* (%) values refer to validated subjects outside the RI. Bold values indicate more than 5% of subjects were outside the 95th percentiles of the RIs.

Abbreviations: AFP, alpha-fetoprotein; CA19-9, cancer antigen19-9; CA15-3, cancer antigen 15-3; CA125, cancer antigen125; CEA, carcinoembryonic antigen; CI, confidence interval; Cyfra 21-1, cytokeratin 19 fragment; F, females; HE4, human epididymis protein 4; M, males; ProGRP, progastrin releasing peptide; PSA, prostate specific antigen; RI, reference interval.

[a]Reference intervals established in this study;

[b]Reference intervals currently used in our laboratory;

[c]Reference intervals provided by the manufacturers.

checkup population. Ozarda et al.[17] recommended that indirect data-mining methods can be applied to a laboratory's existing data to verify RIs established using an external source. Though the RIs established in the present study were derived from our own database using the same analytical method and local population, they were validated using a relatively large number of healthy reference individuals from the health checkup population. Less than 5% of subjects were outside the 97.5th percentiles of all RIs established in the present study. However, more than 5% of subjects were outside the 95th percentiles of RIs for several age and sex subgroups, such as across all age subgroups for PSA. These findings suggest that an upper limit of the 97.5th percentiles of the RIs established in the present study could be more acceptable for use in our laboratory. Meanwhile, less than 3% of the healthy reference subjects fell outside the current and manufacturers' RIs of all tumor markers except Cyfra 21-1, which could be indicative of the RIs being too wide.

This study has some limitations. First, the CLSI recommends using the direct method to establish RIs, whereas an indirect method was used in the present study. Nevertheless, we obtained a large amount of data from a database covering 16 health promotion centers. Unhealthy subjects based on the results of sonography, endoscopy, and radiographic and laboratory tests were excluded from the reference population used to establish RIs in this study. Second, we were not able to establish the RIs for CA125 and HE4 according to the menstrual cycle or menopausal status. Nevertheless, the levels of CA125 and HE4 were determined according to age in the present study, with the RI of HE4 increasing with age and that of CA125 decreasing at ≥50 years of age.

The strengths of this study include the use of the same instruments with an identical analytical method to obtain the results for nine common tumor markers. We analyzed the pooled data of health examinees aged 18–98 years obtained from 16 health promotion centers in 13 cities across Korea. This approach allowed us to identify not only age- and sex-specific RIs of common tumor markers that are representative of the Korean population, but also dynamic changes from early adulthood to the late geriatric age.

5 | CONCLUSION

Age- and sex-specific RIs for nine common tumor markers have been established in the healthy Korean population. These RIs could be useful information for clinicians making clinical decisions and assessments.

AUTHOR CONTRIBUTIONS

Eun-Hee Nah: Conceptualization; data curation; formal analysis; investigation; methodology; supervision; validation; writing—original draft; writing—review and editing. **Seon Cho**: Conceptualization; data curation; formal analysis; investigation; methodology; validation; writing—review and editing. **Hyeran Park**: Conceptualization; investigation; methodology; validation. **Suyoung Kim**: Conceptualization; formal analysis; investigation; methodology; validation. **Eunjoo Kwon**: Conceptualization; data curation; investigation. **Han-Ik Cho**: Conceptualization; methodology; supervision; validation; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

This authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study was reviewed and approved by the Institutional Review Board at the Korea Association of Health Promotion (approval number: 130750-201608-HR-024). This study was a retrospective study of medical records and all data were fully anonymized before authors accessed them and IRB waived the requirement for informed consent.

TRANSPARENCY STATEMENT

The lead author Eun-Hee Nah affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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