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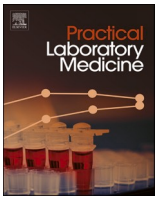

Comparison of results and age-related changes in establishing reference intervals for CEA, AFP, CA125, and CA199 using four indirect methods

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1. Introduction

The incidence of tumors is progressively increasing and becoming a leading cause of death in patients aged <70 [1]. Tumor markers, such as carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), carbohydrate antigen 125 (CA125), and carbohydrate antigen 199 (CA199), find wide application in clinical practice. While these biomarkers lack specificity for malignant tumors, a wealth of clinical evidence has substantiated that elevated concentrations prior to surgery or treatment can offer valuable indications regarding the tumor's status. The reference intervals (RIs) associated with these markers serve as useful diagnostic aids in this regard. Currently, RIs for these markers commonly utilized in laboratories are sourced from documents such as health industry guidelines, textbooks, and instructions provided by instrument or reagent manufacturers. However, geographical, dietary, genetic, sex, and age variations in the population can lead to individual discrepancies. Inappropriately matched RIs for the served population can either heighten patient anxiety and unnecessary testing if too narrow, or delay early diagnosis if too broad. Regular evaluation and validation of these RIs are required according to ISO 15189:2022 [2] and the Clinical and Laboratory Standards Institute (CLSI) EP28-A3 guidelines [3]. Personalized RIs for tumor markers should be established when necessary [4].

Establishing RIs typically involves both direct and indirect methods. Although direct methods are recommended by CLSI and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [5],they need to recruit 120 healthy individuals in each partition (such as sex, age range), which is time-consuming, resource-intensive, and costly [6]. Moreover, achieving optimal results for age- and sex-related factors can be challenging. With the advent of big data, the use of indirect methods to establish RIs is gaining popularity in laboratories [7]. These methods offer the advantages of large datasets, no requirement for reference population recruitment, and easy ethical compliance [6]. The EP28-A3c guidelines include standard parameters or non-parametric statistical methods as indirect approaches [3]. Although they are simple to use and popular in research for setting RIs, they are prone to distortion by extreme data, which are often the most indicative of disease presence. Recent advancements have led to the development of more sophisticated indirect methods based on open-source software algorithms, such as refineR [8], Kosmic [9], and TMC [10], each of which boasts unique algorithms and features, is accessible through open-source code, is compatible with the R language environment, and facilitates ease of implementation.

While the incidence of tumors is relatively low in children, it significantly increases in older adults. This study analyzed three years of medical examination data and each subgroup ($n > 400$) surpassed the minimum number of evaluations recommended by the IFCC Committee on Reference Intervals and Decision Limits [11]. The study aimed to investigate the variations and effects of age and sex on

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Non-standard abbreviations

AFP	alpha-fetoprotein
CA125	carbohydrate antigen 125
CA199	carbohydrate antigen 199
CEA	carcinoembryonic antigen
CLSI	Clinical and Laboratory Standards Institute
CIs	Confidence Intervals
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
Kosmic	Kolmogorov-Smirnov distance
TMC	Truncated minimum card method
MetS	Metabolic syndrome
NPM	Non-parametric method
RIs	Reference intervals

the reference intervals (RIs) for CEA, AFP, CA125, and CA199 through different indirect methods.

2. Materials and methods

2.1. Data sources

The datasets used in this study were obtained from the First Affiliated Hospital of Zhejiang University, located in Hangzhou, Zhejiang Province, eastern China, which experiences a subtropical monsoon climate with distinct seasons. The predominant ethnic group is the Han Chinese. The test dataset included individuals who underwent CEA, AFP, CA125, and CA199 testing during physical examinations between January 2021 and September 2023, we grouped the test dataset by age and sex to establish reference intervals. The validation dataset consisted of individuals who underwent physical examinations and testing for the same items in October 2023, and also grouped the validation dataset by age and sex to validate the relevant test datasets. Both sets predominantly represent Physical examination population for the non manual workers. All data processing and computations were double-checked, and the study was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University (approval number: 2022). (1569).

2.2. Instruments and reagents

The detection instrument used was an Alinity i chemiluminescence immunoassay analyzer (Abbott, USA). The reagents and calibrators used were the original products obtained from Abbott. High-, medium-, and low-level quality control materials were provided by Techno Path (USA). The instrument was regularly calibrated and maintained, and daily quality control was conducted according to the Standard Operating Procedure after control checks. The laboratory was certified by the College of American Pathologists (CAP) and accredited according to ISO 15189.

2.3. Data processing

2.3.1. Data acquisition

Health examination data, including name, sex, birth date, test name, test result, instrument number, and testing time, were retrieved from the laboratory information systems using SQL queries in the PL/SQL Developer and saved in xlsx format.

2.3.2. Data preprocessing

2.3.2.1. Removal of missing values. Records missing birth date or sex information were deleted.

2.3.2.2. Deduplication and anonymization. Duplicate records were identified and removed based on names, sex, and birth date, taking only the first result for multiple entries. The names were anonymized to protect privacy.

2.3.2.3. Handling special characters. Non-numeric results,CEA <0.5 ng/mL, were converted to numeric values 0.5 ng/mL.For values like CA199 $<$ 2 U/mL, random numbers between 0 and 1 were generated using the Excel RAND function and multiplied by 2 to replace missing values. Values of CA199 $>$ 12000 U/mL, were converted to 12000 U/mL.

2.3.3. Grouping

The data, excluding individuals under 18, was divided into 14 subgroups based on sex and age for CEA, AFP, CA125, and CA199, namely M18–29, M30–39, M40–49, M50–59, M60–69, M70–79, M $\geq$ 80, F18–29, F30–39, F40–49, F50–59, F60–69, F70–79, and F $\geq$ 80 (M, males; F, females).

2.3.4 Outlier handling: SPSS 25.0 (IBM,USA) was used for normality testing of CEA, AFP, CA125, and CA199. Because all variables showed a non-normal distribution, the Box-Cox transformation in R (MASS package) was applied to normalize the data. The transformed data were assessed using Tukey method to eliminate high-value outliers. This process was repeated until no outliers were detected, after which the data were returned to their original scale.

2.4. Establishment of RIs

The RIs and 90 % confidence intervals (CIs) for the 14 subgroups of the four biomarkers were calculated using the RefineR, Kosmic, TMC, and non-parametric algorithms. Because the lower limits of tumor markers do not hold clinical significance, only the upper limits, specifically the 95th percentile, were used to establish the RIs.

2.4.1. RefineR method

This method employs a three-step inverse modeling process that begins by determining the parameter search area and peak, followed by a multilevel grid search to find the best model parameters, and finally extracts the RIs from the optimal model [8]. The RefineR algorithm is implemented using the R package 'refineR,' available at https://cran.r-project.org/web/packages/refineR/index.html.

2.4.2. Kosmic method

This algorithm uses power normal distribution modeling. It first applies the Box-Cox transformation to the data, and then fits a Gaussian distribution to the truncated data. The Kolmogorov-Smirnov distance between the truncated observed distribution and the Gaussian distribution was calculated by selecting the smallest distance as the RI of healthy individuals [9]. The Kosmic algorithm is implemented in R using the 'tidykosmic' package, accessible at https://www.divinenephron.co.uk/tidykosmic/.

2.4.3. TMC method

Previously reported by Wosniok and Haeckel, this new indirect method uses the estimated values of the best-fit interval for RIs calculation. It allows for pre-estimation grouping of the population by age and sex, directly outputting the RIs for all age-sex groups and offering a more straightforward visualization for researchers [10]. The TMC algorithm was implemented using the R 'TMC' package, available at https://user.math.uni-bremen.de/c05c/TMC/.

2.4.4. Non-parametric method

The 95th percentile results were calculated to establish the RIs, as per the EP28-A3c guideline.

2.4.5 All the above procedures were performed using R version 4.3.1, with the R packages or codes having been previously validated and reported [12–14].

2.5. Validation of RIs

Data from the validation set were used to validate the RIs, with >60 individuals in each subgroup. The RIs established using the four methods were considered valid if 90 % of the validation set fell within the new RIs [15].

2.6. Statistical methods

Data pre-processing was performed using Microsoft Excel version 2003. Skewness-Kurtosis tests for normality were performed using the SPSS 25.0 (IBM,USA) software, Differences among all variable subgroups were assessed using the Kruskal-Wallis test. Statistical significance was considered for p-values that were less than or equal to 0.05.

The R package 'MASS' was used for Box-Cox transformation to achieve normality, and outliers were eliminated using the Tukey

Table 1
Composition of the datasets (M, male; F, female).

Marker	Total	Remove age and gender deficient individuals	Remove duplicate patients	Excluding individuals aged <18 years	outlier		Outlier median[IQR]		Number of enrolled researchers
					M	F	M	F	
CEA	272,408	516	144,991	104	576	639	9.4 (7.4,11.0)	4.9 (4.2,7.2)	125,582
AFP	257,548	519	131,392	99	939	1,321	10.4 (9.2,12.5)	10.4 (8.2,16.9)	123,278
CA125	197,938	328	102,525	79	338	1,527	34.6 (31.2,42.0)	62.1 (51.8,86.7)	93,141
CA199	263,696	517	136,286	101	439	1,259	64.4 (54.2,87.2)	68.9 (58.8,89.8)	125,094

[a]CEA, carcinoembryonic antigen; AFP, alpha-fetoprotein; CA125, carbohydrate antigen 125; CA199, carbohydrate antigen 199; IQR, interquartile range.

Table 2
Distribution of results of CEA, AFP, CA125, and CA199 grouped by age and sex (M, male; F, female).

Marker	Sex	Age group (Years)	N	Median [IQR]	Kruskal-Wallis test		Sex	Age group (Years)	N	Median [IQR]	Kruskal-Wallis test	
					H	*P*					H	*P*
CEA (ng/mL)	M	19–29	11,648	1.7 (1.2,2.4)	5061.28	<0.001	F	19–29	14,144	1.1 (0.8,1.5)	9362.12	<0.001
		30–39	19,209	1.7 (1.2,2.4)				30–39	18,201	1.1 (0.8,1.6)		
		40–49	13,754	1.9 (1.3,2.7)				40–49	10,608	1.3 (1.0,1.8)		
		50–59	13,058	2.2 (1.5,3.1)				50–59	8,903	1.6 (1.2,2.3)		
		60–69	5,891	2.4 (1.7,3.5)				60–69	4,305	1.9 (1.4,2.6)		
		70–79	2,333	2.7 (2.0,3.7)				70–79	1,707	2.3 (1.7,3.3)		
		≥80	1,071	2.9 (2.2,4.0)				≥80	750	2.9 (2.0,3.9)		
AFP (ng/mL)	M	19–29	11,411	2.4 (1.8,3.2)	2087.28	<0.001	F	19–29	13,689	2.1 (1.5,2.9)	3279.58	<0.001
		30–39	18,784	2.7 (2.0,3.7)				30–39	17,558	2.3 (1.7,3.2)		
		40–49	13,568	2.9 (2.2,4.0)				40–49	10,562	2.5 (1.9,3.6)		
		50–59	12,905	2.9 (2.2,3.9)				50–59	8,829	2.8 (2.0,3.8)		
		60–69	5,851	2.9 (2.2,3.8)				60–69	4,279	2.9 (2.2,4.0)		
		70–79	2,342	2.7 (2.0,3.5)				70–79	1,693	2.7 (2.0,3.7)		
		≥80	1,055	2.5 (1.9,3.3)				≥80	752	2.9 (2.2,3.8)		
CA125 (U/mL)	M	19–29	6,640	10.3 (7.9,13.2)	203.27	<0.001	F	19–29	13,538	16.2 (12.1,21.9)	8254.99	<0.001
		30–39	11,590	10.2 (7.9,13.4)				30–39	16,975	15.7 (11.9,21.1)		
		40–49	7,210	10.1 (7.8,13.2)				40–49	10,398	14.8 (11.2,19.9)		
		50–59	6,087	10.1 (7.8,13.3)				50–59	8,824	10.8 (8.3,14.2)		
		60–69	2,920	10.3 (8.0,13.8)				60–69	4,315	10.3 (8.0,13.3)		
		70–79	1,452	11.1 (8.3,14.6)				70–79	1,667	10.7 (8.3,14.0)		
		≥80	779	12.3 (9.1,16.0)				≥80	746	11.9 (9.1,16.2)		
CA199 (U/mL)	M	19–29	11,672	4.5 (2.4,8.7)	594.19	<0.001	F	19–29	13,874	8.2 (4.1,15.5)	1570.14	<0.001
		30–39	19,222	4.4 (2.4,8.2)				30–39	17,939	7.2 (3.8,13.7)		
		40–49	13,790	4.6 (2.6,8.5)				40–49	10,615	6.6 (3.4,12.5)		
		50–59	13,121	4.8 (2.8,8.7)				50–59	8,924	5.3 (2.8,10.5)		
		60–69	5,899	5.3 (3.1,9.4)				60–69	4,317	4.7 (2.6,9.3)		
		70–79	2,321	5.9 (3.6,10.6)				70–79	1,652	4.6 (2.7,8.9)		
		≥80	1,058	7.1 (4.2,12.1)				≥80	690	5.1 (2.9,8.7)		

[a]CEA, carcinoembryonic antigen; AFP, alpha-fetoprotein; CA125, carbohydrate antigen 125; CA199, carbohydrate antigen 199; IQR, interquartile range.

method. The establishment of the RIs using the four indirect methods was performed using R 4.3.1. Line plots of CIs for these methods were generated using the R package 'ggplot2,' and forest plots the validation were created using 'forest plot' package.

3. Results

3.1. Composition of the datasets

For the four biomarkers CEA, AFP, CA125, and CA199, data were collected and refined by removing duplicates, excluding individuals aged <18 years, and filtering out outlying values.The resulting datasets are listed in Table 1.

3.2. The distribution of results grouped by sex and age

Table 2 illustrates the distribution of CEA, AFP, CA125, and CA199 results categorized by age and sex. The Kruskal-Wallis test was used to evaluate differences across various sex and age groups for all biomarkers, and these differences were statistically significant ($P < 0.05$).

The key findings were as follows: 1) CEA: Both male and female CEA levels increased with age. In males, the level increased from 1.7 to 2.9 ng/mL, and in females, from 1.1 to 2.9 ng/mL 2) AFP: Variations in AFP levels across different age groups and sexes were minimal, with values ranging approximately from 2.1 to 2.9 ng/mL 3) CA125:In Male, CA125 levels increased with age from 10.1 to 12.3 U/mL. In contrast, Female CA125 levels decreased with age, dropping from 16.2 U/mL in the 18–29 years of age group to 10.3 U/mL in the 60–69 years of age group, followed by a gradual increase to 11.9 U/mL in those aged 80 and above. 4) CA199: For males,

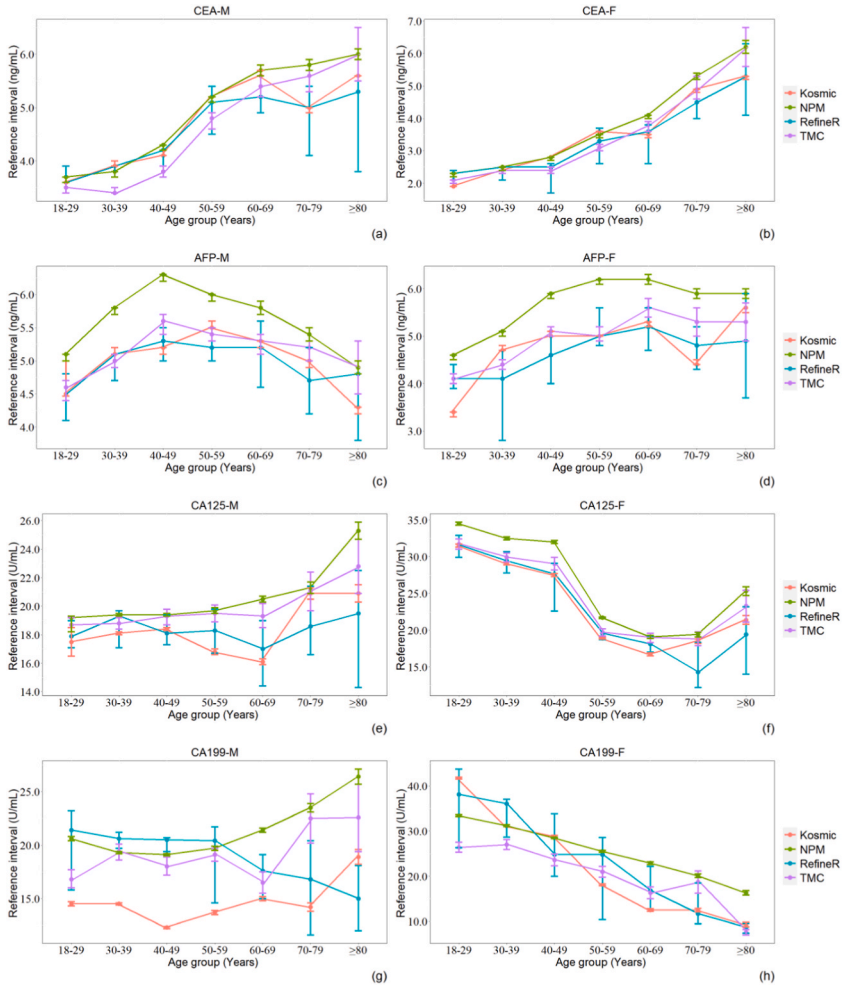

Fig. 1. Comparison of RIs established using four indirect methods

*M, male; F, female; and NPM, non-parametric method. Different colors represent different algorithms, the points on the line represent the reference interval values of the corresponding groups, The horizontal lines at both ends of this point represent the 90 % CIs of this point. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

CA199 levels remained relatively stable in patients aged 18–59 years and then increased with advancing age, peaking at 7.1 U/mL in those aged ≥80 years. Females CA199, however, they decreased with age, from 8.2 U/mL in the 18–29 years of age group to 4.6 U/mL in the 70–79 years of age group, before slightly increasing to 5.1 U/mL in patients aged >80 years.

3.3. Comparison of RIs establishment using four indirect methods

The RIs established using the RefineR, Kosmic, TMC,and non-parametric methods are shown in Fig. 1 (detailed data are available in Appendix A). Different colors represent different algorithms, the points on the line represent the reference interval values of the corresponding groups, The horizontal lines at both ends of this point represent the 90 % CIs of this point.

The non-parametric method yielded broader RIs than the other three methods; however, overall, the trends of the RIs were consistent across all four indirect methods. The broadest ranges for all populations and subgroups were CEA <6.2 ng/mL, AFP <6.3 ng/mL, CA125 < 34.5 U/mL, and CA199 < 41.7 U/mL. The results for CEA and CA125 were largely consistent across all methods; AFP intervals were slightly broader in the non-parametric method, whereas the other three methods showed similar results. For CA199, the trend across all age groups was broadly similar in all four methods, although some variations were observed within specific subgroups.

Key findings are as follows: 1) CEA levels were highest in individuals aged >80 years for both sex (Males, 6.0 ng/mL; Females, 6.2 ng/mL). 2) AFP levels showed minimal variation across sex and age groups, peaking at 6.3 ng/mL in males aged 40–49 years and at 6.2 ng/mL in females aged 60–69 years. 3) CA125 levels were higher in females, peaking at 34.5 U/mL in the 18–29 years of age group, and then decreasing in the 60–69 years of age group. 4) CA199 levels were also higher in females, peaking at 41.7 U/mL in the 18–29 years of age group and decreasing with age, reaching the lowest levels in females aged >80 years.

3.4. Validation of RIs established by the four methods

The validation results for the RIs established using the RefineR, Kosmic,TMC, and non-parametric methods are illustrated in Fig. 2. Each project is stratified by age and sex, and then validated by creating a validation chart. The position of each point on the chart represents the group's pass rate. A red dashed line is utilized as the 90 % pass threshold. The pass rate to the left of the red line ranges from 0.8 to 0.9, while the pass rate to the right of the red line ranges from 0.9 to 1.0. The black dot located to the left of the red dashed line represents the verification failure point, while the point located to the right of the dashed line represents the verification pass point.

Four indirect methods have been validated in both CEA and AFP. For CA125, the intervals passed the validation across all age groups using the TMC and non-parametric methods. For CA199, non-parametric method intervals were validated. However, some sex, age groups did not pass the validation for CA125 and CA199 using the RefineR and Kosmic methods. Specifically as follows: 1) CA125 did not pass validation in RefineR (M70–79, F70–79, and M ≥ 80) and Kosmic (M60–69 and M ≥ 80). 2) CA199 did not pass validation in RefineR (M70–79, F70–79, and F ≥ 80), Kosmic (M40–49, M70–79, F70–79, and F ≥ 80), and TMC (F ≥ 80). The detailed data on these validations are provided in Appendix B.

4. Discussion

This comparison of RIs established using four indirect methods, namely RefineR, Kosmic, TMC, and the non-parametric method, revealed similar trends. Notably, the non-parametric method tends to produce broader intervals, whereas the Kosmic method often

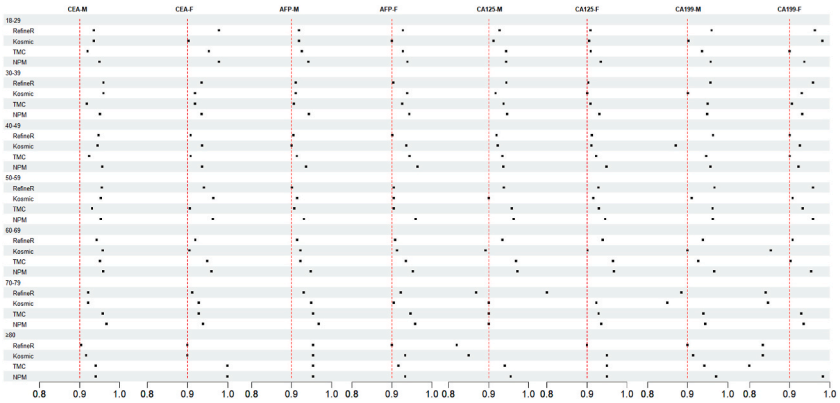

Fig. 2. Comparison of four algorithms validation pass rates in males and females
*M, male; F, female; and NPM, non-parametric method,the position of each point on the chart represents the group's pass rate. A red dashed line is utilized as the 90 % pass threshold. The pass rate to the left of the red line ranges from 0.8 to 0.9, while the pass rate to the right of the red line ranges from 0.9 to 1.0. The black dot located to the left of the red dashed line represents the verification failure point, while the point located to the right of the dashed line represents the verification pass point. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

results in narrower intervals for specific subgroups. A significant distinction lies in the ability to isolate outliers; the non-parametric method does not effectively separate abnormal values and may include data from unhealthy individuals, whereas RefineR, Kosmic, and TMC effectively mitigate the influence of outliers on the results.Studies suggest that RefineR performs optimally with large sample sizes ($n \geq 5000$) and when outliers constitute $\leq$30 % of the data [8]. Kosmic is more effective when the outliers are <20 %, and pre-eliminating outliers can improve the accuracy of RIs [9]. TMC, an iterative method, assumes data from healthy or a minority of diseased individuals and computes the final results using non-pathological interval data during the iteration [10].Therefore, even though the non parametric method has the highest validation pass rate, we prefer to recommend the other three indirect methods to establish RIs.

Despite their complex algorithms, RefineR, Kosmic, and TMC offer user-friendly open-source software packages, simplifying operations for non-experts, and featuring relatively fast computation times. Under the default settings, RefineR and Kosmic can produce results and draw RIs distribution graphs within 5 s. TMC, owing to preset age and sex intervals, operates slightly slower but can output results for different age-sex segments and consolidate them into visualizations within 3 min. However, these methods require users to have basic computing skills to set up an R environment, download R packages, and modify the parameters. Data must be formatted appropriately for each method.However, We found that the distribution of raw data significantly affects the results of the algorithm. For example, in CA199, there are approximately 13000 pieces of data that are less than the lower detection limit of 2U/mL. We have tried to convert them to the same value, but Kosmic cannot produce results, while refineR and TMC can. Therefore, we filled in the data using the Excel rand function, but the filled values may also affect the final result.

Furthermore, it is worth mentioning that the levels of CA125 and CA199 in the female groups of RefineR and Kosmic were observed to be notably low. This could be attributed to the heterogeneity of results between men and women, particularly among older adult women where the data exhibited a more right-skewed distribution. Additionally, it should be noted that there was a relative scarcity of data for older women in the study. So the original data distribution significantly influenced the consistency and accuracy of the algorithm [16–18]. Based on our current usage, the TMC algorithm is more robust, has a higher validation pass rate, and can directly output reference ranges for different ages and sexes.

Most of the RIs we established using four indirect methods were within the existing conventional reference range.Notably, for CA125 and CA199, several age groups exhibited significantly narrower intervals than the standard intervals,as shown in Fig. 1.

As shown in Fig. 1-(a and b), all four indirect methods revealed a noticeable age-related increase in CEA levels in both males and females, which is consistent with the findings of Li et al. [19]. The incidence of metabolic syndrome (MetS) increases with age [20], as demonstrated in a cross-sectional study in a Chinese population, where the prevalence increased from 16 % among individuals aged 18–44 years to 38 % among those aged >65 years [21]. Factors associated with MetS, such as blood sugar, triglycerides, and hypertension, correlated with higher CEA levels [22]. Additionally, CEA levels in males were slightly higher across all age groups, potentially because of the impact of smoking [23]. The smoking rate was significantly higher among Chinese males (50 %) than to females (2 %) [24], which could explain the sex differences in CEA levels.

AFP has little difference among different age and sex groups. These observations were similar to those reported by Nah et al. [25], as shown in Fig. 1-(c and d).

CA125 levels in females were the highest in the 18–29 years of age group, which may be attributed to increased sexual activity during this period, potentially leading to conditions such as endometriosis and other non-cancerous inflammatory gynecological diseases [26,27]. The decline in CA125 levels with age could be related to the unique physiological functions of females, including a gradual decline in ovarian function and hormonal levels with age, resulting in lower CA125 levels. This pattern is consistent with that reported by Park et al. [28]. Additionally,CA125 levels increase after 60 years of age, possibly due to significant changes in estrogen secretion following ovarian atrophy during menopause, which increases the risk of ovarian cancer [29]. Excessively high and low RIs can lead to misdiagnosis or missed diagnoses. However, the reason for this age-related increase in CA125 levels in males requires further investigation, as shown in Fig. 1-(e and f).

Additionally, we have found that female CA199 levels were significantly higher than those in males, peaking in the 18–29 years of age group and showing a notable decline with age. This trend may be linked to menstrual cycles and other hormonal fluctuations in females, consistent with the findings of Zhang et al. [30]. Researches have shown a correlation between CA199 and blood sugar levels in individuals with type 2 diabetes [31]. Therefore, the increase in CA199 levels in males may be related to the accumulation of age-related diseases. In females, however, hormonal influences appear to play a more significant role than aging, as indicated in Fig. 1-(g and h).

5. Limitations

This study has several limitations: it is a cross-sectional study, possibly leading to information bias and limiting insights into longitudinal patient changes; it is a single-center study, which could introduce selection bias; the validation population was randomly selected without strict disease exclusion criteria; and the grouping was solely based on age and sex, without considering statistical differences.

6. Conclusion

After extensive data mining, this study found that the age and sex trends of RIs established by RefineR, Kosmic, TMC, and non-parametric methods for CEA, AFP, CA125, and CA199 were generally similar. However, the TMC algorithm demonstrated a higher relative pass rate, greater robustness, and was determined to be better suited to our laboratory's needs. It should be noted that the

accuracy and consistency of the algorithm can be affected by the distribution of raw data.

CRediT authorship contribution statement

Juping Chen: Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Formal analysis, Data curation. **Lina Fan:** Writing – original draft, Validation, Software, Methodology, Formal analysis, Data curation. **Zheng Yang:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Investigation, Formal analysis, Data curation. **Dagan Yang:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Research funding: The National Key R&D Program of China, Grant number: 2022YFC3602302.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plabm.2023.e00353.

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