

Age- and Sex-Specific Reference Intervals for Laboratory Parameters from Aging Trajectories in Healthy Beijing Adults (2010–2026)

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Abstract

Background

Current reference intervals (RIs) applied uniformly across ages ignore physiological aging.

Aims

We aimed to characterize age-dependent biomarker trajectories, identify aging inflection points, and establish age- and sex-stratified reference intervals in apparently healthy Chinese adults.

Methods

We analyzed laboratory data from 4,392 healthy adults (1,894 males, 2,498 females; aged 18–91 years) across four survey waves (2010–2026). A three-tier non-circular exclusion framework (infectious disease serology, cross-system extreme-value thresholds, and refineR statistical decomposition) was applied. Inflection points were detected via segmented regression with bootstrap validation. RIs (P2.5–P97.5) were derived nonparametrically with 90% bootstrap CIs and validated using the 2024 elderly cohort.

Results

Four consensus inflection points (~ 35, 50, 60, and 70 years) were identified; the 50–55 year peri-menopausal inflection in women was the most consistent. Age-stratified RIs deviated substantially from uniform RIs: 42 of 160 (26.3%) indicator-sex-age groups had out-of-range (OOB) rates > 20%, with CHOL in women aged 50–59 years reaching 58.8%. Cross-year validation showed new RIs achieved closer-to-target OOB rates in 60.9% of groups. Significant secular trends included GGT + 35% and UA + 17% over 16 years, while LDL declined 18%; anchor indicators (MCV, MCH) remained stable (< 2% change).

Discussion

Our non-circular, age-differentiated exclusion framework avoids reproducing existing reference intervals and prevents physiological aging from being misclassified as pathology, supporting age-adapted interpretation.

Conclusions

Uniform RIs systematically misclassify age-related physiological changes, particularly for lipids in peri- and post-menopausal women. Our age- and sex-specific RIs, grounded in aging trajectories, support periodic recalibration and age-adapted interpretation to reduce misclassification and improve clinical decision-making.

1. Introduction

Reference intervals (RIs) are fundamental tools in clinical decision-making, defining the range within which 95% of results from a healthy reference population fall [1,2]. However, most RIs in clinical use in China derive from young to middle-aged adults or from manufacturer specifications and are applied uniformly regardless of age[3]. This "one-size-fits-all" approach ignores the well-documented physiological changes that accompany aging across organ systems [4,5].

Aging is not linear but a complex, multidimensional process[6]. Chinese population-based studies have identified physiological aging inflection points—around 38, 49, 69, and 77 years in one community cohort [7], and 30 and 50 years in a multi-omics cohort of Chinese women [8]—and Wang et al. showed that 25 of 36 routine biomarkers had U-shaped, nonlinear associations with mortality [9]. Laboratory parameters therefore undergo systematic, predictable age-related change.

If biomarkers change predictably with age, then RIs derived from young adults will systematically misclassify physiologically normal age-related changes as pathological—or mask true pathology against an inappropriate reference background. This is especially pressing for China, which faces the world's most rapid demographic aging alongside profound nutritional and lifestyle transitions [10,11].

The CLSI EP28-A3c guideline recommends partitioning RIs by factors that contribute to clinically significant variation, including age and sex [1]. A fundamental methodological challenge remains: how should "healthy" be defined without circularly relying on the very RIs one aims to establish?

To address these challenges, we analyzed laboratory data from 4,392 apparently healthy Chinese adults across four survey waves (2010–2026) with three aims: (1) to characterize age-related biomarker trajectories and inflection points; (2) to establish age- and sex-stratified RIs using a non-circular exclusion framework; and (3) to evaluate secular trends to inform periodic RI recalibration.

2. Methods

2.1 Study Population and Data Source

This study analyzed de-identified laboratory data from four community-based health-survey waves of apparently healthy adults undergoing routine check-ups at a tertiary hospital in Beijing: 2010 (n = 1,098), 2014 (n = 2,047), 2024 (n = 878), and 2026 (n = 779). Inclusion required self-reported good health and absence of major chronic disease, long-term medication, recent surgery, blood loss, smoking, or alcohol

use, with normal cognition and no clinically significant electrocardiographic abnormality; pregnant and lactating women were excluded. Participant flow is shown in Supplementary Fig. 1.

2.2 Three-Layer Non-Circular Exclusion Framework

A fundamental challenge in RI establishment is avoiding circular reasoning: if individuals are excluded based on existing RI cutoffs, the newly derived RIs will inevitably reproduce those same cutoffs [12]. We addressed this through a three-layer non-circular framework:

Layer 1—Disease-state exclusion: Individuals with positive serology for hepatitis B surface antigen ($n = 92$) or anti-hepatitis C virus antibody ($n = 23$) were excluded ($n = 115$)—definitive diagnoses independent of any RI under investigation.

Layer 2—Cross-system orthogonal extreme-value exclusion: Fifteen criteria spanning six organ systems were defined at disease-diagnostic thresholds rather than reference-interval limits (Supplementary Table 1). Key thresholds included serum glucose (GLU) > 15.0 or < 2.5 mmol/L (uncontrolled diabetes or severe hypoglycemia; American Diabetes Association crisis thresholds [13]); creatinine (Crea) > 200 $\mu\text{mol/L}$ for adults < 60 years (overt renal impairment, $\text{eGFR} < 30$ mL/min/1.73 m²; KDIGO stage-4 threshold [14]); triglycerides (TG) > 10.0 mmol/L (severe hypertriglyceridemia with pancreatitis risk [15]); γ -glutamyl transferase (GGT) > 300 U/L, aspartate aminotransferase (AST) > 200 U/L, and total bilirubin (TBIL) > 50 $\mu\text{mol/L}$ (each > 5 -fold above the upper reference limit); white blood cell count < 2.0 or $> 15.0 \times 10^9/\text{L}$; platelet count < 50 or $> 600 \times 10^9/\text{L}$; uric acid > 600 $\mu\text{mol/L}$; urea > 12 mmol/L; and urine-dipstick abnormalities. To avoid circularity, criteria were applied orthogonally—when deriving RIs for a target organ system, only criteria from other systems were activated.

For participants ≥ 60 years, creatinine (> 200 to > 250 $\mu\text{mol/L}$) and hemoglobin thresholds (men: < 90 to < 80 g/L; women: < 80 to < 70 g/L) were relaxed to avoid misclassifying age-appropriate changes—declining glomerular filtration and mild anemia—as pathology [14,16,17]. A total of 290 individuals were flagged by Layer 2 criteria (280 net exclusions after removing those already excluded by Layer 1); the complete criteria are in Supplementary Table 1.

Layer 3—Statistical decomposition: Following clinical exclusion, the refineR algorithm [18] was applied to each biomarker within each age-sex stratum to statistically separate the presumed healthy subpopulation distribution from any residual pathological signal, using Box-Cox transformed inverse modeling.

2.3 Laboratory Parameters

The following biomarkers were analyzed and grouped by organ system: (1) hepatic parameters: alanine aminotransferase (ALT), AST, GGT, alkaline phosphatase (ALP), TBIL, direct bilirubin (DBIL), total protein (TP), albumin (ALB); (2) renal parameters: Crea, UA, Urea; (3) lipid parameters: TG, cholesterol (CHOL), high density lipoprotein (HDL-C), low-density lipoprotein (LDL-C); (4) glycemic parameters: GLU; and (5) hematological parameters: WBC, red blood cell (RBC), HGB, hematocrit (HCT), PLT, mean corpuscular

volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC).

2.4 Detection of Age Inflection Points

For each of the 24 parameters, segmented regression was fitted to the 48 biomarker–sex combinations. Biomarkers were retained if either Criterion A ($|\text{Spearman } \rho| > 0.20$ in either sex, capturing monotonic trends) or Criterion B (segmented-regression $F > 4$, detecting nonlinear inflections) was met. These two criteria capture complementary signals: Criterion A detects monotonic trends but is insensitive to a symmetric inverted-U trajectory, which yields $\rho \approx 0$ despite a strong biological signal; Criterion B fills this gap. Requiring both simultaneously would exclude biomarkers with the strongest inflections (e.g., CHOL, $F = 236.6$, $\rho = 0.148$) and those with the strongest linear trends (e.g., ALB, $\rho = 0.503$, $F = 20.1$); the OR rule therefore retains both trajectory types.

Twenty-three biomarkers met at least one criterion; one (MCHC) was excluded for weak age correlation. Of the 46 combinations for the 23 retained biomarkers, 43 showed robust breakpoints ($F > 4$) and informed consensus inflection detection.

A two-segment piecewise linear model ($y = b_0 + b_1 \cdot \text{age} + b_2 \cdot \max(0, \text{age} - \text{bp})$) was fitted for each biomarker–sex combination, with the breakpoint (bp) selected by grid search (25–85 years) minimizing residual sum of squares. Improvement over a linear model was assessed by F-test, BIC, and percentage reduction in RSS, with breakpoint stability confirmed by bootstrap resampling ($n = 500$).

Change-point analysis: As a complementary method, the Pruned Exact Linear Time (PELT) algorithm [19] with an L2 cost function was applied to age-binned (2-year intervals) median values.

Consensus inflection points were identified by kernel density estimation on the weighted distribution of robust breakpoints (weighted by F-statistic). Given the cross-sectional design, "trajectory" refers to population-level age-associated patterns rather than within-individual change.

2.5 Establishment of Reference Intervals

The four consensus inflection boundaries (~ 35 , ~ 50 , ~ 60 , and ~ 70 years) identified in Section 3.2 were applied uniformly to define five age strata (18–34, 35–49, 50–59, 60–69, and ≥ 70 years) for all biomarkers, regardless of whether an individual biomarker contributed to inflection detection. These boundaries thus serve as a common age-partitioning framework for reference interval derivation.

For each biomarker, sex, and age stratum with at least 120 observations, RIs (P2.5–P97.5) were established nonparametrically in accordance with CLSI EP28-A3c [1,20], with 90% confidence intervals from 1,000 bootstrap iterations; for strata with 40–119 observations, the robust (Tukey biweight) method was used.

2.6 Cross-Year Validation

The 2024 survey wave (n = 774) comprised exclusively elderly participants (59–91 years), serving as an independent validation set for RIs derived from the training waves (2010, 2014, 2026; n = 3,618). Validation required that the out-of-range percentage (OOB%) fall closer to the nominal 5% target with the new RIs than with existing RIs. Because the validation set included only elderly participants, validation in younger age groups remains pending.

2.7 Secular Trend Analysis

To distinguish biological secular trends from analytical drift, we used ANCOVA (age-adjusted, year as categorical predictor), Spearman correlation between year and OOB rate within strata, and "anchor indicators" (MCV, MCH)—erythrocyte indices expected to be insensitive to calendar time. Analyses used Python 3.12 (NumPy, SciPy, pandas, statsmodels) and R 4.5 (refineR, segmented, mgcv).

3. Results

3.1 Study Population Characteristics

After the three-layer cleaning framework, the analytic sample comprised 4,392 individuals (1,894 men [43.1%], 2,498 women [56.9%]; median age 55 years, range 18–91), distributed across 2010 (n = 1,039), 2014 (n = 1,885), 2024 (n = 774), and 2026 (n = 694) waves; all sex-stratified age subgroups exceeded 290 individuals (Supplementary Table 2). The 2024 wave comprised only older adults (59–91 years).

3.2 Aging Trajectories and Inflection Points

Applying the dual-threshold criteria to all 24 parameters, 23 met at least one criterion and were retained (all organ systems represented); one (MCHC) failed both criteria. The 16 biomarkers with well-established existing reference intervals formed the core set for reference-interval derivation and are shown in Fig. 1.

LOESS-smoothed age trajectories for the 16 core biomarkers, with segmented-regression breakpoints overlaid, are presented in Fig. 1. Three distinct aging trajectory patterns emerged:

Pattern A—Monotonic change: Urea, UA, TG, GLU, MCV, and MCH increased progressively across the adult lifespan in both sexes, while TP and DBIL declined. Biomarkers with weak net age dependence (AST, HDL-C, WBC; $|p| < 0.20$) were not assigned a pattern.

Pattern B—Midlife peak or trough with slope reversal: ALT (male: peak at 34 years, slope + 0.85 to – 0.32 U/L per year, $F = 84.5$; female: peak at 56 years, + 0.28 to – 0.18, $F = 77.8$), GGT (male: peak at 36 years, + 1.37 to – 0.32, $F = 105.4$; female: peak at 53 years, + 0.32 to – 0.01, $F = 23.0$), CHOL (male: peak at 52 years, $F = 168.5$; female: peak at 55 years, $F = 236.6$), LDL-C (male: peak at 39 years, $F = 124.7$; female: peak at 55 years, $F = 219.6$), ALP (female: peak at 67 years, + 0.78 to – 0.55, $F = 102.9$), PLT (male: peak at 41 years, $F = 24.3$; female: peak at 43 years, $F = 48.9$), HGB (female: peak at 61 years, + 0.13 to – 0.30, $F = 72.4$), HCT (female: peak at 62 years, $F = 79.1$), and RBC (female: peak at 61 years, $F = 106.3$) rose

from early adulthood to midlife and declined thereafter, forming inverted U-shaped trajectories. TBIL exhibited a U-shaped trough in both sexes (male 38 years; female 47 years), dipping in early middle age before rising.

Pattern C—Late-life acceleration of pre-existing trend: Several biomarkers were stable through early and middle adulthood before a marked slope change at advanced age (breakpoint ≥ 55 years). Creatinine in men showed an accelerated increase after 63 years (slope -0.12 to $+0.64$, $F = 87.6$); HGB in men remained stable until 66 years, after which the decline rate increased 5.5-fold (slope -0.12 to -0.66 , $F = 46.5$); ALB declined with acceleration after ~ 61 years in both sexes (male: -0.07 to -0.14 , $F = 20.1$; female: -0.03 to -0.12 , $F = 52.5$); HCT in men declined faster after 66 years ($F = 45.0$); RBC in men declined with acceleration after 48 years ($F = 35.0$); and UA in men transitioned from gradual decline to late-life rise after 67 years (slope -0.62 to $+1.22$, $F = 11.7$). These late-life inflections, absent or attenuated in younger groups, reflect progressive loss of physiological reserve across hematopoietic, renal, hepatic, and musculoskeletal systems.

Median percentage change from the youngest (18–34) to oldest (≥ 70) group revealed striking sex differences. In women, TG increased 103.4%, ALP 40.4%, GGT 41.7%, ALT 33.3%, Urea 31.7%, LDL-C 18.0%, and UA 17.6%, whereas DBIL declined 35.7% and PLT 12.8%. In men, Urea increased 22.9%, TG 17.7%, and TBIL 15.5%, while DBIL declined 25.8%, ALT 19.0%, PLT 17.9%, ALB 9.7%, RBC 9.6%, and ALP 6.6%.

Robust breakpoints ($F > 4$) were identified for 43 of 46 biomarker–sex combinations. Kernel density estimation of weighted breakpoints revealed six peaks ($\sim 28, 36, 47, 55, 60, 66$ years) consolidated into four boundaries: $\sim 35, \sim 50, \sim 60$, and ~ 70 years (Fig. 1).

3.3 Age- and Sex-Stratified Reference Intervals

Of the 24 parameters, 16 had well-established existing RIs enabling formal out-of-range comparison; the remaining eight (DBIL, TP, ALB, RBC, HCT, MCV, MCH, MCHC) lacked widely accepted sex-specific RIs and were therefore excluded from the out-of-range analysis.

A total of 160 age- and sex-stratified RIs (P2.5–P97.5) were established across the 16 biomarkers; the complete table with 90% confidence intervals is in Fig. 2 and Supplementary Table 3.

Several notable patterns emerged from the stratified RIs. The ALT upper limit declined substantially with age in men: from 82.9 U/L (18–34 years) and 83.0 U/L (35–49 years) to 51.0 U/L (50–59 years), 42.0 U/L (60–69 years), and 45.6 U/L (≥ 70 years), contrasting sharply with the uniform existing RI of 9–50 U/L. In women, the CHOL upper limit rose markedly at midlife: 6.24 mmol/L (18–34 years), 6.90 mmol/L (35–49 years), peaking at 7.66 mmol/L (50–59 years), then 7.40 mmol/L (60–69 years) and 7.32 mmol/L (≥ 70 years), far exceeding the existing 5.2 mmol/L. GGT in men showed a pronounced midlife peak (112.3 U/L at 35–49 years), nearly double the existing 60 U/L.

3.4 Inadequacy of Existing Uniform Reference Intervals

Out-of-range percentages (OOB%) for healthy individuals under existing uniform RIs were evaluated across all 160 biomarker–sex–age combinations. Mean OOB was 14.4% (median 9.6%)—nearly three times the 5% target—with 42 combinations (26.3%) exceeding 20% and four above 50%. Lipid parameters in middle-aged and elderly individuals were most affected (Table 1, Fig. 3).

Table 1
Top 10 biomarker-sex-age groups with the highest out-of-range rates using existing uniform RIs.

Rank	Biomarker	Sex	Age Group	OOB%	Existing RI
1	CHOL	F	50–59	58.8	2.8–5.2
2	CHOL	F	60–69	54.5	2.8–5.2
3	LDL	F	50–59	52.0	0-3.4
4	CHOL	F	70+	51.0	2.8–5.2
5	CHOL	M	50–59	47.4	2.8–5.2
6	LDL	F	60–69	42.9	0-3.4
7	CHOL	M	35–49	41.4	2.8–5.2
8	TG	M	35–49	41.4	0.56–1.7
9	CHOL	M	60–69	40.1	2.8–5.2
10	LDL	M	35–49	39.5	0-3.4

Misalignment was systematic (Supplementary Fig. 2): CHOL and LDL exceeded the 5% target in nearly all but the youngest age groups; GLU and Crea deviated most in the oldest groups; whereas the existing ALT upper limit (50 U/L) was overly lenient for older men, classifying only 2.5–4.1% as above-range despite a true P97.5 of 42–46 U/L.

3.5 Cross-Year Validation

In the independent 2024 elderly validation cohort (n = 774), the new age-stratified RIs achieved a mean OOB of 8.2% versus 14.9% for existing uniform RIs (Table 2), and were closer to the nominal 5% target in 39 of 64 (60.9%) validation groups.

Table 2
Cross-year validation results using the independent 2024 elderly cohort.

Metric	New RIs	Existing RIs
Mean OOB rate	8.2%	14.9%
Mean within-RI coverage	91.8%	85.1%
Groups closer to 5% target	39/64 (60.9%)	19/64 (29.7%)
Groups with OOB < 10%	47/64 (73.4%)	33/64 (51.6%)

The greatest improvements were CHOL in women aged 60–69 (OOB 47%→17%), TG in men ≥ 70 (44%→15%), LDL in women 60–69 (31%→6%), and CHOL in women ≥ 70 (50%→22%). The residual 8.2% OOB reflects genuine inter-individual variation and the difficulty of estimating extreme percentiles from finite samples—underscoring that RI refinement is iterative.

3.6 Secular Trends in Population Biochemistry

Over 16 years (2010–2026), ANCOVA revealed significant age-adjusted year effects ($p < 0.05$) in 21 of 24 biomarker–sex combinations (87.5%); mean partial eta-squared was 0.056, indicating year explained ~ 5.6% of variance after accounting for age .

Anchor indicators changed minimally: MCV (+ 1.7% male, + 0.8% female) and MCH (– 0.4% male, – 1.5% female), arguing against major analytical drift but not excluding secular changes in unmeasured confounders. Secular-trend interpretations (Supplementary Table 4) are speculative and unadjusted for adiposity.

4. Discussion

4.1 Principal Findings

This study of 4,392 apparently healthy Chinese adults across four survey waves (2010–2026) provides comprehensive evidence that existing uniform RIs are systematically inadequate for age-stratified interpretation. Four principal findings emerged.

First, we identified four consensus aging inflection points (~ 35, ~ 50, ~60, ~ 70 years), detected by segmented regression with bootstrap validation and independently corroborated by PELT change-point analysis. The ~ 50-year inflection, coinciding with the female perimenopausal transition, was the most robust and consistent across biomarkers. These align with and extend prior work: the Jiangxi community study identified inflections at 38.2, 49.4, 68.9, and 76.9 years [7], and a Chinese female multi-omics study reported major transitions at 30 and 50 years [8]. While statistically robust, the clinical utility of certain inflection-based splits (e.g., the 35- and 70-year boundaries for ALT) warrants prospective outcome

validation, as the absolute slope differences at these breakpoints, though significant, are modest in magnitude.

Second, age- and sex-stratified RIs differed substantially from uniform RIs, with 26.3% of combinations exceeding 20% OOB—most severely for lipid parameters in peri- and post-menopausal women (OOB 51–59%), reflecting loss of estrogen-mediated cardiometabolic protection [22,23]. A postmenopausal woman with cholesterol 6.5 mmol/L—within her healthy peers' central 95%—would be labeled "hypercholesterolemic," whereas an elderly man with ALT 48 U/L would be labeled "normal" despite exceeding his age-specific 97.5th percentile (42 U/L).

Third, cross-year validation confirmed the robustness of the new RIs, achieving OOB rates closer to the target than existing RIs in 60.9% of validation groups.

Fourth, we documented significant 16-year secular trends (GGT + 35%, UA + 17%, LDL – 18%). Given medication screening, the LDL decline likely reflects dietary and lifestyle changes in this health-conscious, medication-naïve subpopulation [10,21,24]; stable anchor indicators (MCV, MCH) argue against major analytical drift.

4.2 Methodological Contribution: The Non-Circular Exclusion Framework

A key methodological contribution is the three-layer non-circular exclusion framework: definitive disease-state labels (infectious serology), cross-system orthogonal extreme-value criteria set at disease-diagnostic rather than RI thresholds, and statistical decomposition via refineR. Differential age-specific Layer 2 thresholds further prevented misclassifying physiological aging as pathology—a critical, often overlooked consideration in geriatric laboratory medicine [25].

4.3 Clinical Implications

For lipid management: the 5.2 mmol/L CHOL limit labels roughly half of apparently healthy peri- and post-menopausal women "abnormal"; age- and sex-stratified RIs would reduce false-positive dyslipidemia labeling and its treatment, anxiety, and resource implications.

For hepatic assessment: the ALT upper limit declines markedly with age in men (83 to 42 U/L); age-stratified limits would help avoid missing mild hepatic injury—including non-alcoholic fatty liver disease—in older adults whose ALT appears "normal" by young-adult standards.

For renal monitoring: late-life acceleration of Crea and UA, and accelerated HGB decline after 66 years, support more frequent renal and hematopoietic monitoring after the seventh decade.

The observed 18% decline in LDL-C over 16 years in this medication-naïve cohort merits comment. Globally, non-HDL cholesterol has risen markedly in East Asia over recent decades, repositioning China as a central locus of the global cholesterol burden [21]; within Beijing, dietary surveys indicate energy from fat rose from 31.6% to 36.9% between 2010 and 2022, with a progressive shift from plant- to

animal-sourced fat [10]. Against this backdrop, the LDL-C decline observed here suggests the strictly screened, apparently healthy subpopulation follows a divergent trajectory. Three non-mutually-exclusive explanations warrant consideration. First, individuals who self-select into health surveys and meet stringent exclusion criteria may be disproportionately health-conscious, adopting practices—such as replacement of saturated with unsaturated fats [24]—that diverge from population averages. Second, secular improvement in cooking-oil quality, specifically the progressive replacement of lard and palm oil with soybean and rapeseed oils in Chinese households, may have shifted the fatty-acid profile toward a more favorable composition. Third, exclusion of medication use selects individuals whose lipid trends are shaped entirely by non-pharmacological factors; the LDL decline may therefore represent the net effect of countervailing dietary and lifestyle changes unmasked by the removal of pharmacological noise. Regardless of mechanism, lipid secular trends in a well-characterized healthy cohort differ from general-population estimates [21], underscoring the value of rigorous reference-population definition and the need for periodic RI recalibration.

4.4 Limitations

Limitations include the repeated cross-sectional design, which precludes longitudinal inference; possible residual subclinical disease; and the 2024 elderly-only validation, which leaves younger-age RI performance unconfirmed. Absence of BMI data precluded adiposity adjustment, and different survey waves may have involved different analytical platforms despite anchor-indicator stability—standardized anthropometric and inter-laboratory quality-control protocols would strengthen future surveys. Medication exclusion limits generalizability to a medication-naïve population, and modest subgroup sizes, while meeting CLSI minima [1], constrain extreme-percentile precision—multi-center validation in larger, medication-diverse cohorts is warranted.

Inflection points rest on statistical criteria alone; outcome-based studies must establish clinical utility. The elderly-relaxed Layer 2 thresholds may inadvertently retain individuals with mild renal impairment—sensitivity analyses with alternative cutoffs would quantify this trade-off. Given ongoing secular trends, periodic recalibration of these RIs is warranted.

4.5 Conclusion

Age- and sex-stratified reference intervals, derived by physiological aging-trajectory analysis, represent a clinically meaningful refinement over uniform RIs. The four inflection points (~ 35, ~ 50, ~60, ~ 70 years) provide a physiologically grounded age-stratification framework, and the elevated out-of-range rates with current uniform RIs—especially for lipids in middle-aged and elderly women—underscore the urgency of adoption. Documented secular trends further highlight the need for periodic recalibration. Future work should validate these findings in longitudinal cohorts with outcome data and evaluate the clinical impact of age-stratified RI adoption.

Declarations

Competing interests:

The authors have no competing interests to declare that are relevant to the content of this article.

Ethics approval:

This study was approved by the Medical Science Research Ethics Committee of Peking University Third Hospital (approval number: M20241021).

Artificial intelligence disclosure

The authors did not use artificial intelligence or large language model tools in the preparation of this manuscript.

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Author Contribution

Q. L., JJ. T., and H. W. contributed equally to this work and wrote the main manuscript text and performed the formal data analysis. C. W., BX. Y., and Z. X. performed data curation and statistical validation. YY. N., ZX. L., ZJ. S., XY. X., and HK. G. contributed to clinical sample collection, data acquisition, and interpretation. S. Y. and LY. C. conceptualized and supervised the study, revised the manuscript critically, and provided research funding. All authors reviewed the manuscript, commented on previous versions, and read and approved the final manuscript.

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Data availability:

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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Figures

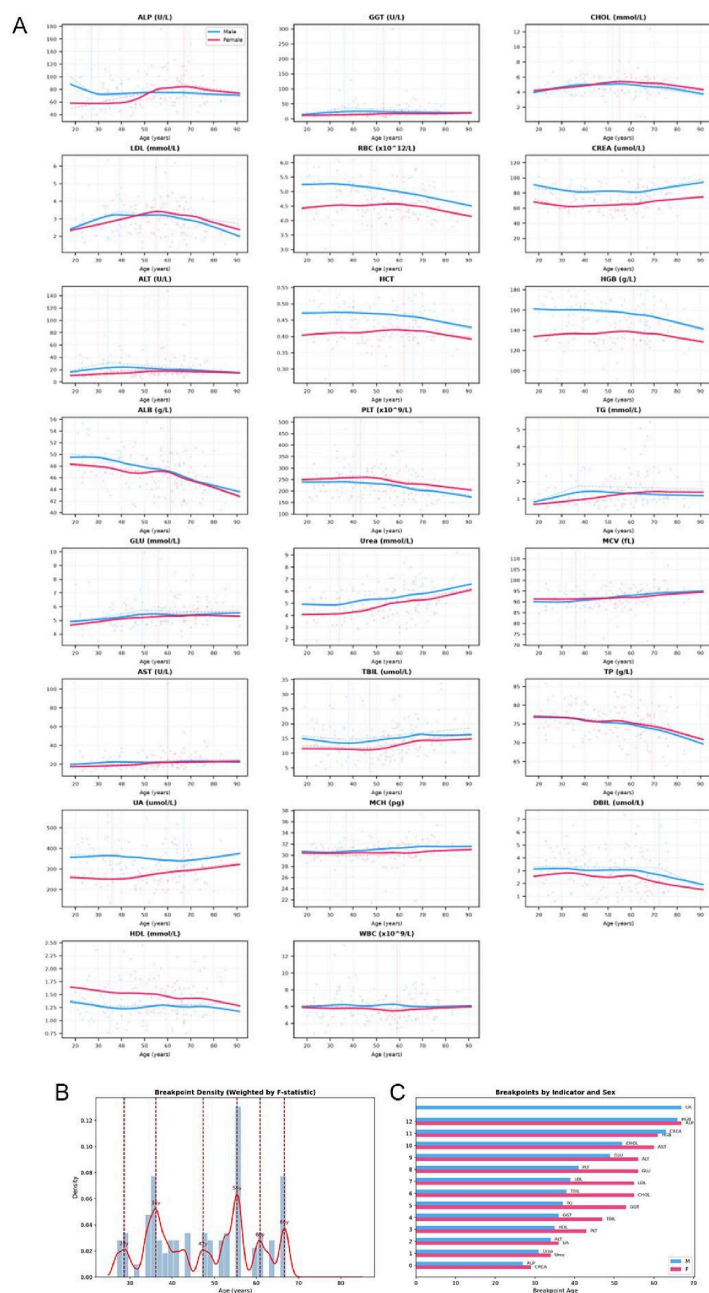


Figure 1

Age-dependent biomarker trajectories, consensus inflection zones, and individual breakpoints.

(A) Segmented regression curves for 16 common laboratory parameters (each biomarker by sex) across age (18–91 years) in apparently healthy Chinese adults. (B) Kernel density estimate (KDE) of all weighted breakpoints (weighted by F-statistic) from all biomarker–sex combinations. (C) Individual

breakpoints for each biomarker–sex pair, sorted by breakpoint age and color-coded by sex (blue for males, pink for females).

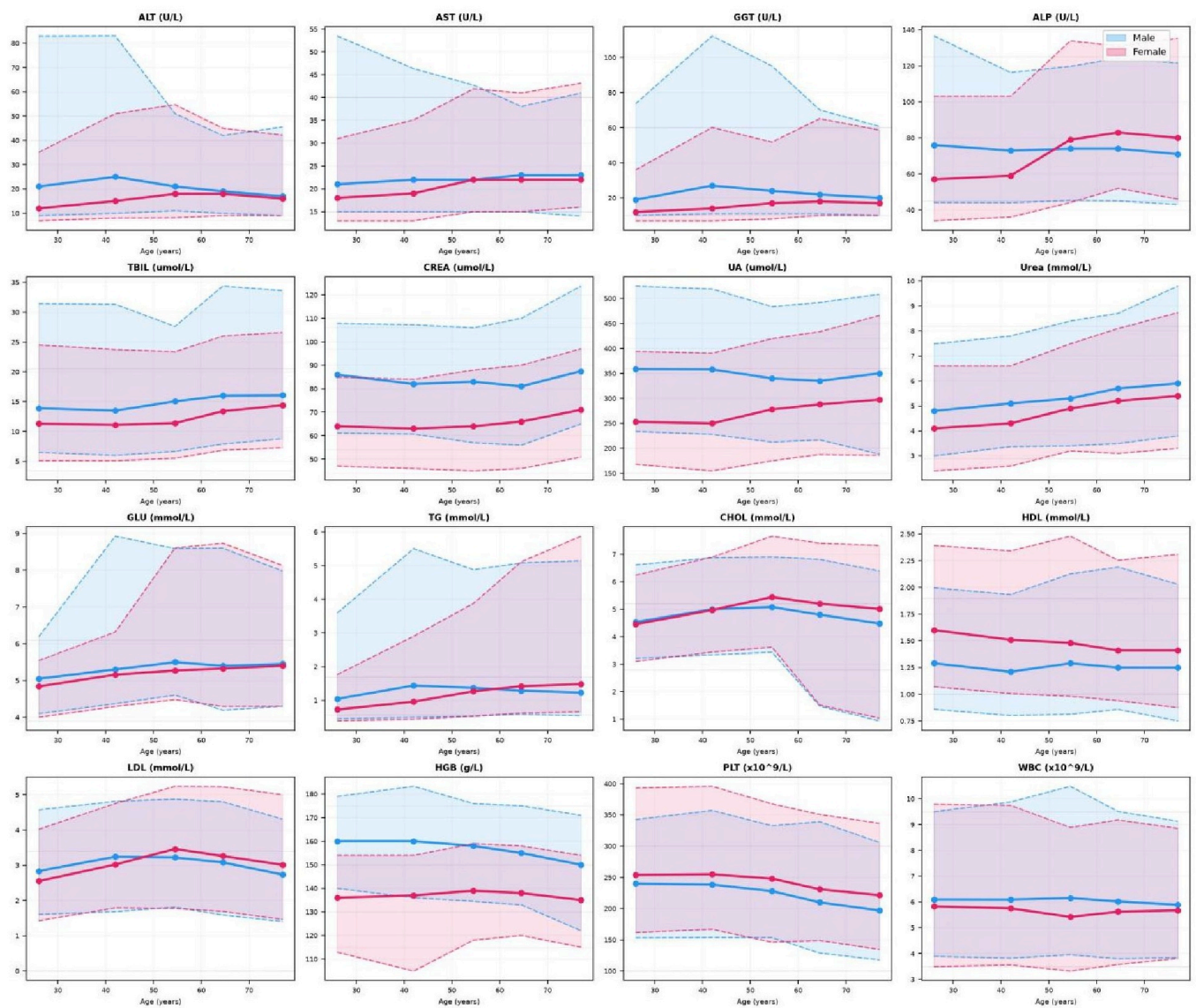


Figure 2

Age- and sex-specific reference intervals for All 16 Biomarkers with Existing Reference Intervals.

Shaded bands represent the P2.5-P97.5 nonparametric reference interval. Solid lines connect median values across age groups. Dashed horizontal lines indicate the existing uniform RI for each sex. The width and central tendency of the RI change systematically with age.

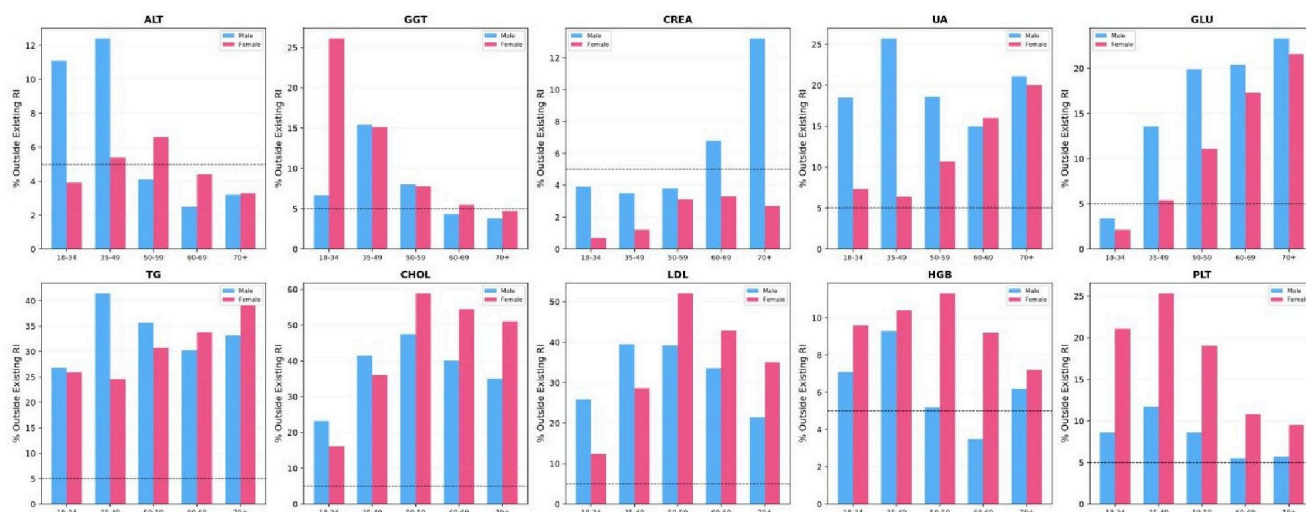


Figure 3

Proportion of healthy individuals falling outside existing uniform reference intervals, by age group and sex.

Supplementary Files

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