Review

Age-Adjusted Reference Values for Prostate Specific Antigen – A Systematic Review and Meta-Analysis

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Abstract

Objectives: To systematically evaluates the evidence on ethnic differences in age-adjusted reference values of PSA. **Materials and Methods:** In concordance with the Preferred Reporting Items for Systematic Review and Meta-analysis statement, a review of English articles using Medline, Embase and Cochrane databases, from inception to December 2019 was conducted. Studies that reported the PSA upper reference value as 95th percentile of the cohort distribution, in healthy men aged 40 to 79, were included. Methodological quality was assessed with a modified version of the Agency for Healthcare Research and Quality checklist for cross-sectional studies. **Results:** Forty-three studies examining 325,514 participants were included in the analysis. These were published between 1993 and 2018. Majority were prospective observational studies and reported the reference values in ten-year age intervals. Only five reports directly compared ethnic differences in PSA values. Due to missing data, six studies were not considered in the quantitative synthesis. For the remainder (37/43), heterogeneity in PSA reference values was considerable (Higgin's index = 99.2%), with age and ethnicity being the sole identified significant contributors. Accordingly, the pooled upper limits for PSA reference values were 2.1, 3.2, 4.9 and 6.5 ng/ml for men in their 40 s, 50 s, 60 s, and 70 s, respectively. **Conclusion:** Moderate quality evidence suggest that upper PSA reference limits increased with age and significant ethnic differences were present.

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Introduction

Prostate-specific antigen (PSA) is a commonly utilised serum biomarker for the early detection of prostate cancer (Pca), and for surveillance and disease progression.[1,2] The validity of PSA-based methods as screening tools for Pca, is an ongoing controversial topic. Several attempts have been made to improve the accuracy of Pca diagnosis using other structural forms of PSA, such as free PSA and pro-PSA; adjusting with PSA velocity and density; as well as recent developments in other novel biomarkers. Nevertheless, interpretation of total serum PSA levels remains a readily available tool in daily clinical practices to aid in the Pca diagnostic decisions.

Since the introduction of PSA in screening, a cut-off level of 4.0 ng/ml has been widely considered as an abnormal value, beyond which further investigations were necessary to rule out Pca.[3-5] However, the accuracy of this cut-off for Pca has been questionable, since PSA levels tend to be raised with non-oncological conditions, such as benign prostatic hyperplasia and prostatitis.[6] Moreover, in healthy men, it is evident that PSA increases normally with age.[4,7] Therefore, the concept of age-adjusted reference ranges, with upper and lower limits determined as the 95th and 5th percentiles, have been proposed to improve the PSA predictive accuracy for Pca.[4,7-10]

Alongside with age, previous reports have suggested the presence of ethnic differences in PSA levels, which further questions the applicability of a single cut-off value in urological practices.[11-14] However, the effects of ethnicity and age on PSA level variations have not been systematically appraised in the literature.

This study aims to review the literature to assess the variabilities in age-adjusted reference values for healthy men, across different ethnicities and populations, with meta-analyses performed to derive a pooled international age-specific upper reference limit.

Materials and Methods

Eligibility Criteria

This review has been registered with PROSPERO (CRD42021225027) and conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-

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Analyses" (PRISMA) statement.[15] It was approved for ethics by the Health and Disability Ethics Committee (HDEC reference number 17/CEN/239). We investigated all studies that reported age-adjusted PSA reference values in healthy men. This was restricted to published full text articles in English. This restriction was to avoid the absence of peer-review element in unpublished literature. Participants were healthy men, aged 40 to 79 years, with no evidence of Pca. Studies that reported an upper limit for PSA reference values, defined as the observed or estimated 95th percentile of the entire cohort's PSA measurements, were included. Studies that did not report these reference values, either numerically or graphically, in age groups of 10 years or less, were excluded.

Information Sources

Studies were identified by searching electronic databases, scanning reference lists of relevant articles, and consultations with experts in the field. The only limit applied was on language, to consider solely English articles. Using the Ovid platform, a comprehensive search of Embace and Medline databases, from inception until December 2019, was performed. Moreover, the Cochrane library was inspected for any previous pertinent reviews. Supplementary approaches to identify studies included hand searching of articles' reference lists, and consulting with expert personnel from multiple facilities that offer PSA testing locally and internationally.

Search Strategy

The literature search plan was developed, and conducted by two authors, with the aid of experienced library team at their institution. For both Medline and Embace databases, the following keywords were used, with all the subheadings included: Prostate specific antigen, Reference values. Those terms were linked with "AND", with a selected limit to "English Language". The identified records were exported, with complete references, into a separate library on EndNote (by Calrivate Analytics), for management.

Study Selection

After removing the duplicates, two study team members (first and second authors), assessed the eligibility of each record independently, in a non-blinded standardized manner. The first step in the assessment was by title and abstract screening of the citation in a chronological order. For studies that advanced beyond this stage, full text and reference assessment was conducted. The infrequent disagreements between the reviewers, were resolved by consensus.

Data Collection Process

A standard data extraction sheet was developed to include the necessary information. This sheet was pilot tested on randomly selected five included articles. To reduce the bias during the data collection, two reviewers extracted the information separately. This was followed by each reviewer double-checking the other's data extract. Extra attention was implemented when authors had multiple studies attached to their names, in order to avoid the repetitive inclusion of the same study population. Graphical data were converted to numerical using "Web Plot Digitizer", a web-based tool that has a proven reliability in performing such tasks.[16] If data were missing or further information was required, serious attempts were made to contact the corresponding authors to request for further information.

Data Items

The information collected from each study were: (a) study characteristics (author, year, settings, type of study, inclusion and exclusion criteria and recruitment timeframe); (b) participants characteristics (number, age and ethnicity); (c) PSA testing methods in the laboratory; (d) outcome variables including age-adjusted reference value, mean, median, standard deviation (Sd), 95% confidence intervals (CI) and standard errors (Se).

Methodological Quality of Individual Studies

Two independent team members evaluated each study across several domains, by relying on information presented in the main article, or any supplementary reports. The assessment used the Agency for Healthcare Research and Quality methodological checklist for cross-sectional studies.[17] This assessment tool included an 11-point questionnaire to explore the quality of patient recruitment, outcome measurement, potential confounders and follow-up of patients. Each item of the checklist was scored with "Yes," "No," or "Unclear", with one-point allocated for each "Yes". A study was considered of high quality when scored $\geq$7, medium quality when scored 4 to 6, and low quality when scored $\leq$3 points. Disagreements between reviewers were solved by consensus. There was no blinding to authors or journals.

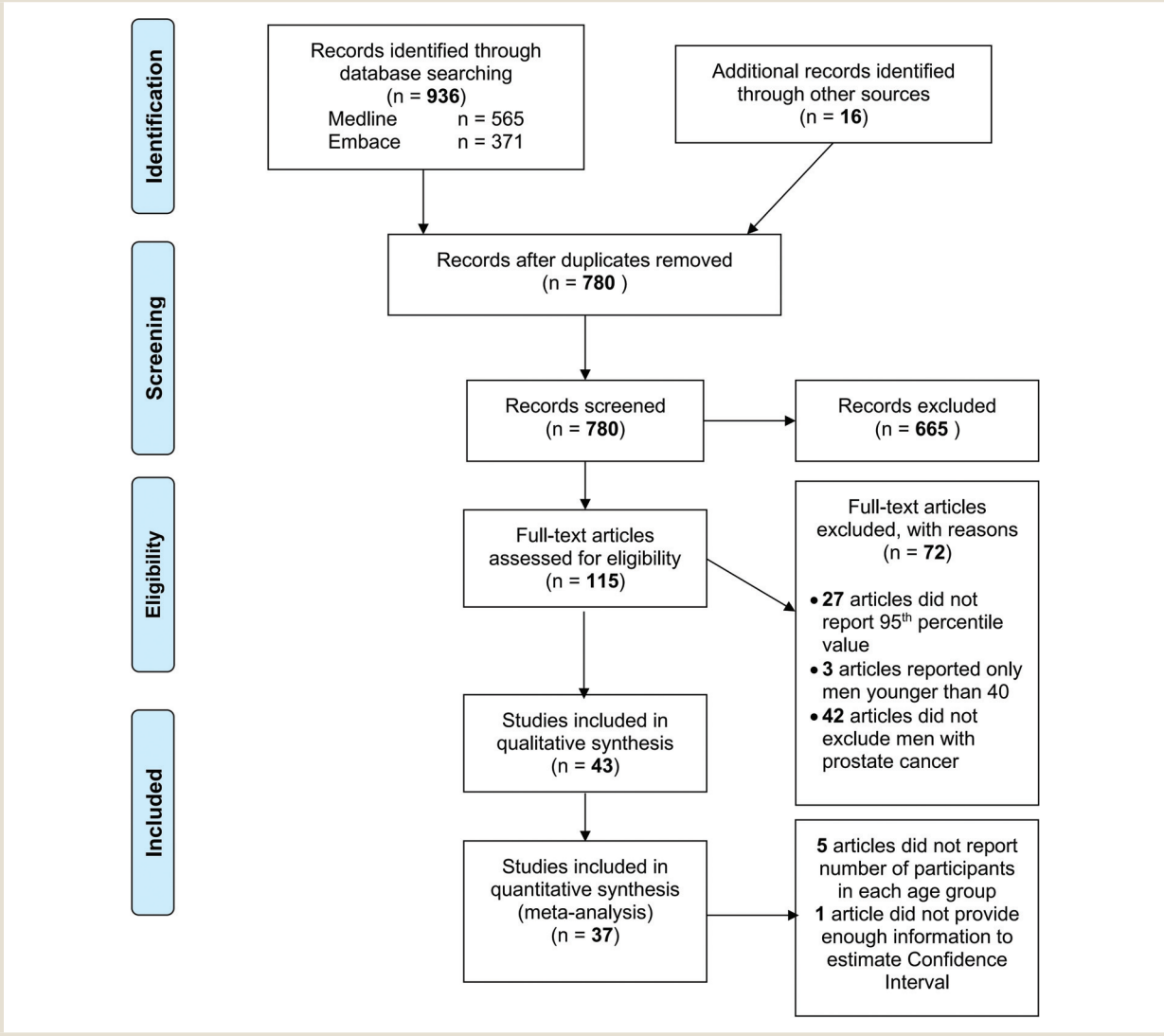
Summary Measures and Planned Methods of Analysis

The outcomes of interest were to investigate the presence of differences in the upper limits of PSA reference values among the studied populations, and to use the most significant factors in constructing pooled age-adjusted values. The analysis was conducted using the METAFOR package in R software version 3.4.4.[18] PSA data were analyzed on the logarithmic scale due to the log normal distribution.

In all studies, due to the nature of the 95th percentile calculations, there were no corresponding estimates of variances. Therefore, the variance was calculated using the asymptomatic formula.[19] Accordingly, this was only possible for studies that provided sufficient information on sample size and either median or mean with Sd. In few cases, the Sd was calculated from the Se or CI.[20] The decision tree for the calculations with the utilized formulas is provided in supplementary material appendix A.

The degree of heterogeneity between the studies was assessed using the Higgins index (I^2) and inspection of forest plots. We assessed both methodological (study settings, quality, PSA analysis technique, outcome measurement method) and clinical factors (age, ethnicity) as possible contributors (moderators) to the heterogeneity in univariable mixed-effect regression model, with amount of residual heterogeneity estimated using the residual maximum-likelihood estimator[21], and the null hypothesis tested with Cochran's Q test. To find the best multivariable model, we compared the single moderator analysis with multiple moderators using single and two-way interaction terms.[22] A stepwise approach (add or drop a term at each step) was used to find the model with the minimum value for the Akaike Information Criterium (AIC).

Figure 1 The PRISMA flow diagram displaying the selection of studies and reasons for exclusion.



Risk of Bias Across Studies

The possibility of publication bias was assessed by evaluating a funnel plot of the residual differences from the mean age adjusted reference value (on the log scale), against the Se. These were evaluated both as an overall, and according to the methodological quality of the included studies. Since graphical evaluation can be subjective, an Egger's test was also conducted to assess the level of symmetry within the plots.[23]

Results

Study Selection

A flow diagram depicting the search and selection of studies is shown in Figure 1. The search of databases revealed 936 records. Sixteen additional articles were identified through the reference screening (manual search). Following removal of duplicates and abstract screening, 115 records were subject to full text review. Seventy one records were excluded as they did not meet the eligibility criteria, which left 43 articles for the qualitative assessment.[4,7-14,24-57] The number of participants for each age group, were not reported in 5 articles[9,25,43,44,52], and the Sd could not be estimated in one article.[48] Therefore, these six studies were excluded from the quantitative synthesis.

Study Characteristics

A description of the main characteristics for each of the 43 included studies is listed in Table 1 . The first published report was in 1993 and the last was in 2018.[4,57] All but five of the reports [9,12,14,46,57] were prospective cohort observational studies, with recruitment time (when reported) ranging from one month to ten years. Eight studies were performed in multiple centres.[12,14,24,29,36,42,45,54] The total sample size in all the studies was 325,514 men (range 236-120,439). The most frequent ethnic

Table 1 Characteristics of the Individual Studies

Author, Year	Study settings[a]	Sample size	Ethnicity/RaceAge (Years)	PSA testing method (Assay type, name, producer)[a b]	Biopsy indication or Exclusion criteria[c]	Main objective	Outcome age interval	Outcome measure-ments method
Oesterling, 1993	USA, single centre, prospective over 2 years	537	White 40-79	IRMA, Tandem-R, Hybritech	PSA >4.0 ng/ml, Suspicious DRE/TRUS	Define age-adjusted reference values for PSA	10 y	Estimated from statistical model
Imai, 1994	Japan, single centre, prospective over 1 y	1480	Japanese 39-89	IRMA, E-test II Kit, Tosoh Co	PSA >6.0 ng/ml, Suspicious DRE/TRUS	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Anderson, 1995	USA, single centre, prospective over 1 mo	1716	Mixed 40-79	MEIA, Imx, Abbott	PSA >4.0 ng/ml, Suspicious DRE	Define age-adjusted reference values for PSA	5 y	Observed PSA values
Bangma, 1995	Netherlands, single centre, retrospective analysis	1762	Europeans 55-76	MEIA, Imx, Abbott and FIA, Prostatus, Wallac	PSA ≥4.0 ng/ml, Suspicious DRE	Prediction accuracy of age-adjusted reference values for prostate cancer	5 y	Observed PSA values
Blijenberg, 1995	International, multicentre, prospective over 2 y	1574	International NR	ELISA, Enzymun-Test, Boehringer	PSA ≥4.0 ng/ml	Evaluation of new PSA testing technique	10 y	Observed PSA values
Oesterling, 1995	Japan, single centre, prospective over 2 years	286	Japanese 40-79	MEIA, Imx, Abbott	PSA >4.0 ng/ml, Suspicious DRE/TRUS	Define age-adjusted reference values for PSA	10 y	Estimated from statistical model
DeAntoni, 1996	USA, multicentre, retrospective analysis	71766	Mixed 40-79	MEIA, Imx, Abbott	PSA >4.0 ng/ml, Suspicious DRE	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Lin, 1996	China, single centre, prospective over 3 years	1008	Chinese 21-80	IRMA, ELSA-PSA2, Isotopen Diagnostick CIS	PSA >4.0 ng/ml, Suspicious DRE/TRUS	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Morgan, 1996	USA, single centre, prospective over 4 years	3475	Black and White 40-79	MEIA, Imx, Abbott	PSA >4.0 ng/ml, Suspicious DRE	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Sawyer, 1996	USA, multicentre, retrospective analysis	10808	Black and White 40 or above	IRMA, Tandem-R, Hybritech	History of Prostate cancer	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Tay, 1996	Singapore, single centre, prospective over 1 month	236	Asian/Chinese 50-88	NR	PSA >4.0 ng/ml, Suspicious DRE	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Kao, 1997	Taiwan, single centre, prospective, timeframe NR	414	Chinese 14-84	IRMA, PSA-RIACT, Isotopen Diagnostick CIS	History of Prostate surgery or cancer	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Lofman, 1997	Sweden, single centre, prospective over 3 mo	878	Swedish 56-75	MEIA, Imx, Abbott	PSA >4.0 ng/ml, Suspicious DRE	Define age-adjusted reference values for PSA	5 y	Estimated from statistical model
Gustafsson, 1998	Sweden, single centre, prospective over 1.5 years	1717	Swedish 55-70	IRMA, Tandem-R, Hybritech	PSA ≥10.0 ng/ml, Suspicious DRE	Define age-adjusted reference values for PSA	5 y	Estimated from statistical model

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Table 1 (*continued*)

Author, Year	Study settings[a]	Sample size	Ethnicity/RaceAge (Years)	PSA testing method (Assay type, name, producer)[a b]	Biopsy indication or Exclusion criteria[c]	Main objective	Outcome age interval	Outcome measure-ments method
Lein, 1998	International, multicentre, prospective over 3 mo	1160	International 20-89	ELISA, Enzymun-Test, Boehringer	History of Prostate surgery or cancer	Define age-adjusted reference values for PSA	10 y	Estimated from statistical model
Chautard, 1999	France, single centre, prospective over 9 months	1274	French 20-69	MEIA, AxSYM, Abbott	PSA >4.0 ng/ml, Suspicious DRE	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Kalish, 1999	USA, single centre, prospective over 2 y	983	Mostly white 48-79	MEIA, AxSYM, Abbott	PSA >4.0 ng/ml	Define age-adjusted reference values for PSA	10 y	Estimated from statistical model
Wolff, 1999	Germany, single centre, prospective, timeframe NR	697	German 20-79	CLIA, Immulite, DPC Biermann	Clinical evidence of Prostate cancer PSA, DRE, TRUS	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Lee, 2000	South Korea, single centre, prospective over 2 years	5801	Korean 30-79	IRMA, ELSA-PSA2, Isotopen Diagnostick CIS	PSA >4.0 ng/ml, Suspicious DRE	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Saw, 2000	Singapore, single centre, prospective, timeframe NR	513	Mostly Chinese 18-89	MEIA, AxSYM, Abbott	History of Prostate cancer	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Cooney, 2001	USA, single centre, prospective, timeframe NR	350	Black 40-79	MEIA, AxSYM, Abbott	PSA >4.0 ng/ml, Suspicious DRE	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Cheli, 2002	USA, multicentre, prospective over 3 years	7541	Mixed 20-94	MSA, Immuno 1, Bayer Diagnostics	Suspicious DRE, PSA >4.0 ng/ml for first study year then age-adjusted PSA cut-off for second and third years	Age-specific values for complexed PSA	10 y	Observed PSA values
Ku, 2002	South Korea, single centre, prospective over 2 years	8297	Korean 20-79	IRMA, Tandem-R, Hybritech	PSA >4.0 ng/ml, Suspicious DRE	Define age-adjusted reference values for PSA	10 y	Estimated from statistical model
Berger, 2003	Austria, single centre, prospective over 2 years	10267	Austrians 40-79	MSA, Immuno 1, Bayer Diagnostics	Suspicious DRE, free PSA <18%, age-adjusted PSA cut-off	Age-specific values for complexed PSA	10 y	Observed PSA values
Gray, 2003	New Zealand, single centre, prospective over 2 years	1405	Multiple 40-69	ECLIA, Cobas core, Roche Diagnostics	PSA >4.0 ng/ml, Suspicious DRE	Define age-adjusted reference values for PSA	10 y	Estimated from statistical model
Kamal, 2003	Saudi Arabia, single centre, prospective, timeframe NR	567	Saudi 40-89	MEIA, NR, NR	PSA >4.0 ng/ml, Suspicious DRE	Define age-adjusted reference values for PSA	10 y	Observed PSA values
He, 2004	China, single centre, prospective over 2 years	1096	Chinese 23-85	NR, ELSA-PSA, E&E Labs	PSA >4.0 ng/ml, Suspicious DRE	Define age-adjusted reference values for PSA	10 y	Observed PSA values

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Table 1 (*continued*)

Author, Year	Study settings[a]	Sample size	Ethnicity/RaceAge (Years)	PSA testing method (Assay type, name, producer)[a b]	Biopsy indication or Exclusion criteria[c]	Main objective	Outcome age interval	Outcome measure-ments method
Kobayashi, 2005	Japan, multicentre, prospective over 2 y	1520	Japanese NR	IRMA, Tandem-R, Hybritech	History of Prostate surgery or cancer	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Muezzinoglu, 2005	Turkey, single centre, prospective, timeframe NR	255	Turkish 40-81	CLIA, ACS:180, Ciba Corning Diagnostics	Clinical evidence of Prostate cancer PSA, DRE, TRUS	Define age-adjusted reference values for PSA	10 y	Estimated from statistical model
Bosch, 2006	Netherlands, single centre, prospective over 10 years	1462	Europeans 50-78	ELISA, Tandem-E, Hybritech and CLIA, Access, Beckman Coulter	PSA >4.0 ng/ml, Suspicious DRE	Define age-adjusted reference values for PSA	5 y	Estimated from statistical model
Choi, 2007	South Korea, multicentre, prospective over 7 years	120439	Korean 30-79	MEIA, AxSYM, Abbott and CLIA, ARCHITECT, Abbott and ECLIA, Cobas core, Roche Diagnostics	History of Prostate surgery or cancer, PSA >10.0 ng/ml	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Ganpule, 2007	India, single centre, cross-sectional study	1899	Indians NR	IRMA, NR, DSL Texas	PSA >4.0 ng/ml, Suspicious DRE	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Khezri, 2009	Iran, single centre, prospective over 2 years	766	Iranian 50-79	ELISA, CanAg, Dianova Sweden	PSA >4.0 ng/ml, Suspicious DRE/TRUS	Define age-adjusted reference values for PSA	10 y	Estimated from statistical model
Liu, 2009	China, single centre, prospective over 3 years	8522	Chinese 18-96	ECLIA, Cobas core, Roche Diagnostics	PSA >4.0 ng/ml, Suspicious TRUS	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Lin, 2010	Taiwan, single centre, prospective over 1 year	7803	Taiwanese 20 or older	NR	PSA >4.0 ng/ml, Suspicious DRE/TRUS	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Yuan, 2011	China, single centre, prospective over 7 years	9358	Chinese 30-79	ECLIA, Cobas core, Roche Diagnostics	PSA >4.0 ng/ml, Suspicious DRE	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Bakir, 2012	Syria, single centre, prospective, timeframe NR	2893	Syrian 40-80	IRMA, Immunotech, Beckman Coulter	PSA >4.0 ng/ml, Suspicious DRE	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Casey, 2012	Ireland, single centre, Prospective over 24 hours	660	Irish 18-67	MEIA, Imx, Abbott	Clinical evidence of Prostate cancer DRE	Define age-adjusted reference values for PSA	5 y	Estimated from statistical model
Liu, 2013	China, single centre, prospective over 1 year	1572	Chinese 40-91	ECLIA, Cobas core, Roche Diagnostics	PSA >4.0 ng/ml, Suspicious DRE/TRUS	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Sarma, 2014	USA, multicentre, prospective, timeframe NR	766	Black and White 40-82	MEIA, AxSYM, Abbott	History of Prostate surgery or cancer	Change in PSA values overtime	10 y	Estimated from statistical model
Ikuerowo, 2016	Nigeria, single centre, prospective, timeframe NR	4035	Nigerian 40-70	CLIA, Access, Beckman Coulter	Suspicious DRE, LUTS †, PSA > 20 ng/ml	Define age-adjusted reference values for PSA	10 y	Observed PSA values

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Table 1 (*continued*)

Author, Year	Study settings[a]	Sample size	Ethnicity/RaceAge (Years)	PSA testing method (Assay type, name, producer)[a b]	Biopsy indication or Exclusion criteria[c]	Main objective	Outcome age interval	Outcome measure-ments method
Yang, 2017	China, single centre, prospective, timeframe NR	1862	Chinese 21-94	ECLIA, Cobas core, Roche Diagnostics	History of Prostate disease or cancer	Define age-adjusted reference values for PSA	10 y	Observed PSA values
Nan, 2018	China, single centre, retrospective analysis	24194	Chinese 30-89	CLIA, LIASON-XL, DiaSorin	PSA > 4.0 ng/ml, free PSA <16%, PSAD > 0.15 †, Suspicious TRUS	Define age-adjusted reference values for PSA	10 y	Observed PSA values

[a] NR = not reported
[b] CLIA = chemiluminescent immunoassay; FIA = fluorescence immunoassay; ECLIA = electro-chemiluminescent immunoassay; ELISA = enzyme-linked immunosorbent assay; IRMA = immuno-radiometric assay; MAS = magnetic separation assay; MEIA = microparticle enzyme immunoassay
[c] DRE = digital rectal examination; LUTS = lower urinary tract symptoms; PSAD = PSA density; TRUS = trans rectal ultrasound.

occurrence was Chinese with ten reports. USA and European countries had 9 and 8 reports, respectively. The participants in all cohorts were apparently healthy males, aged 14 to 96 years, with no evidence of Pca.

The information on the PSA measurement type were ether identified directly from the reports, or cross-referenced from the manufacturers as listed by Semjonow et al[58] Overall, two studies did not report any information on how PSA serum samples were analysed.[25,49] Of the reminder, the two most common methods used were Microparticle Enzyme Immunoassay (14 reports) and Immuno Radiometric assay (11 reports). In more recent years, the Chemiluminesence Immunoassay became more prevalent.

Most studies (33/43) reported detailed information about the criteria for identifying and excluding men with Pca.[4,7-13,24,25,27,28,30,31,33,35-41,44-51,53,55,57] The most common theme was an abnormal (suspicious) Digital Rectal Examination (DRE) findings and a total serum PSA value greater than 4.0 ng/ml, as selection criteria that warrants further investigations (prostate biopsy). The second most used utility was an abnormal Transrectal Ultrasound Scan (TRUS). Conversely, only three studies implemented diagnostic adjuncts including age-adjusted PSA cut-offs, free PSA, and PSA density (PSAD).[36,38,57]

Outcome Measurement Characteristics

Majority (38/43) of the studies had their main objective listed as defining age-adjusted reference values for PSA in healthy men (Table 1).[4,7,8,10-14,25-35,37,39-53,55-57] 5-year and 10-year age group intervals were used in 6 and 37 reports, respectively. Thirty studies calculated the 95th percentile for each group based on the observed PSA values[7-10,12-14,24-26,30,32-36,38,40-42,45,46,48-51,53,55-57], while 13 reports estimated these values from statistical models.[4,11,27-29,31,37,39,43,44,47,52,54] In three reports, the reference values were only provided in graphical format.[25,44,56]

Methodological Quality Assessment

Quality of the included studies varied and none of them fulfilled all 11 quality criteria (supplementary materials appendix B). On average, the total score was 4.4 (range 1-9). Most studies (31/43) thoroughly reported the participants' recruitment methods.[4,8,10-13,24-31,33,36-39,41,42,44-47,49,50,52-54,57] However, only four studies adequately described how missing data were handled in the outcome analysis.[4,11,13,35] Overall, seven studies were judged to be of high methodological quality.[4,13,24,28,35,44,45]

Results of Individual Studies

The reported age-adjusted upper limits of PSA reference values for each study are illustrated in Table 2 . The reference values seemed to increase with each age group. For men in their 40s, these values ranged from 1.23 to 4.78 ng/ml. For men in their 70s, the range was between 3.39 and 15.45 ng/ml. For age group 50 to 59 and 60 to 69, the smallest reference value was reported in a study from France (2.07 and 2.82 ng/ml, respectively)[30], while the greatest were observed in a study of Black men in the USA (6.5 and 11.3 ng/ml, respectively).[13] Five studies directly compared differences in reference values between several ethnic groups, four from USA[12-14,54] and one from New Zealand.[39] The former reports frequently demonstrated that Black men have significantly higher age-adjusted values when compared to White.[12-14,54] Conversely, the New Zealand report did not demonstrate a clear difference between the investigated ethnic groups (European, Māori, and Pacific men).[39]

Results from Meta-Analysis

For the 37 studies included in the quantitative synthesis of results, strong evidence of heterogeneity was observed (I^2 = 99.2%, P < .001). However, the funnel plot did not show signs of asymmetry (supplementary material appendix C) and Egger's test result was not statistically significant (z = - 0.95, P = .341). Of the investigated possible contributors to heterogeneity, only age and ethnicity demonstrated statistically significant effect (Table 3 and supplementary material appendix D).

In multivariable analysis, combining "Grouped ethnicity" and "10-year age intervals" yielded the best model (AIC = 251.2, Figure 2) This model could explain approximately 13% of the total

Table 2 Upper Limit for Age-Adjusted PSA Reference Values in Individual Studies

Author, Year	Ethnicity Reported	Grouped[b]	Age Group (Years)[a]							
			40-49		50-59		60-69		70-79	
			40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79
Oesterling, 1993	USA White	USA White	2.5		3.5		4.5		6.5	
Imai, 1994	Japanese	Japanese	1.3		3.7		4.1		5.1	
Anderson, 1995	USA (mostly white)	USA White	1.3	1.7	2.2	2.9	3.7	4.9	6.4	8.4
Bangma, 1995	Netherlands	European	2.8		3.5	4	4.7	5.4	6.3	
	Netherlands	European	3.1		3.8	4.3	4.9	5.5	6.3	
Blijenberg, 1995	International	International	2		3		4		4.5	
Oesterling, 1995	Japanese	Japanese	2		3		4		5	
DeAntoni, 1996	USA White	USA White	2.3		3.8		5.6		6.9	
	USA Black	USA Black	2.7		4.4		6.7		7.7	
	USA Latino	USA Latino	2.1		4.3		6		6.6	
	USA Asian	USA Asian	2		4.5		5.5		6.8	
Lin, 1996	Chinese	Chinese	2.59		3.31		5.03		5.73	
Morgan, 1996	USA White	USA White	2.1		3.6		4.3		5.8	
	USA Black	USA Black	2.4		6.5		11.3		12.5	
Sawyer, 1996	USA White	USA White	2.01		4.19		7		9.4	
	USA Black	USA Black	2.8		5.4		9.59		15.45	
Tay, 1996	Asian	Asian	N/A	N/A	3.51		3.78		6.02	
Kao, 1997	Chinese	Chinese	1.88		2.37		4.82		5.86	
Lofman, 1997	Swedish	European	N/A	N/A	N/A	4.6	4.4	7.6	8.4	N/A
Gustafsson, 1998	Swedish	European	N/A	N/A	N/A	5.2	5.8	6.7	7.24	N/A
Lein, 1998	European	European	1.75		2.27		3.5		4.26	
Chautard, 1999	France	European	1.33		2.07		2.82		N/A	N/A
Kalish, 1999	USA	USA mixed	N/A	N/A	2.96		6.59		8.84	
Wolff, 1999	German	European	1.4		2.4		4.26		4.69	
Lee, 2000	Korean	Korean	2		2.4		3.9		6.3	
Saw, 2000	Singaporean	Singaporean	1.73		2.25		4.05		6.3	
Cooney, 2001	USA Black	USA Black	2.36		3.27		3.8		5.59	
Cheli, 2002	USA	USA mixed	1.81		2.45		3.17		3.57	
Ku, 2002	Korean	Korean	2.36		2.96		3.78		7.49	
Berger, 2003	Austria	European	1.94		3.5		6.4		8.8	
Gray, 2003	NZ European	European	2.2		2.7		4.2		N/A	N/A
	Maori	Pacific people	2.1		2.6		4		N/A	N/A
	Pacific	Pacific people	2.2		2.8		4.3		N/A	N/A
Kamal, 2003	Saudi	Middle eastern	2.85		3.99		5.41		6.29	
He, 2004	Chinese	Chinese	1.23		2.35		3.2		3.39	
Kobayashi, 2005	Japanese	Japanese	4.7		5.6		11		9.8	
Muezzinoglu, 2005	Turkey	Middle eastern	4.51		4.36		6.17		10.18	
Bosch, 2006	Netherlands	European	N/A	N/A	2.5	3.4	4.7	6.9	N/A	N/A
Choi, 2007	Korean	Korean	1.92		2.37		3.56		5.19	
Ganpule, 2007	Indian	Indian	2.1		3.4		4.2		5	
Khezri, 2009	Iranian	Middle eastern	N/A	N/A	2.61		3.59		4.83	
Liu, 2009	Chinese	Chinese	2.15		3.2		4.1		5.37	
Lin, 2010	Taiwanese	Taiwanese	2.17		3.33		5.11		6.24	
Yuan, 2011	Chinese	Chinese	2.19		2.88		4.42		6.52	
Bakir, 2012	Syrian	Middle eastern	1.7		2.3		4.8		5.8	
Casey, 2012	Ireland	European	1.85	2.17	2.63	3.25	4.02	4.96	N/A	N/A
Liu, 2013	Chinese	Chinese	1.57		2.92		4.11		5.56	

(continued on next page)

Table 2 (*continued*)

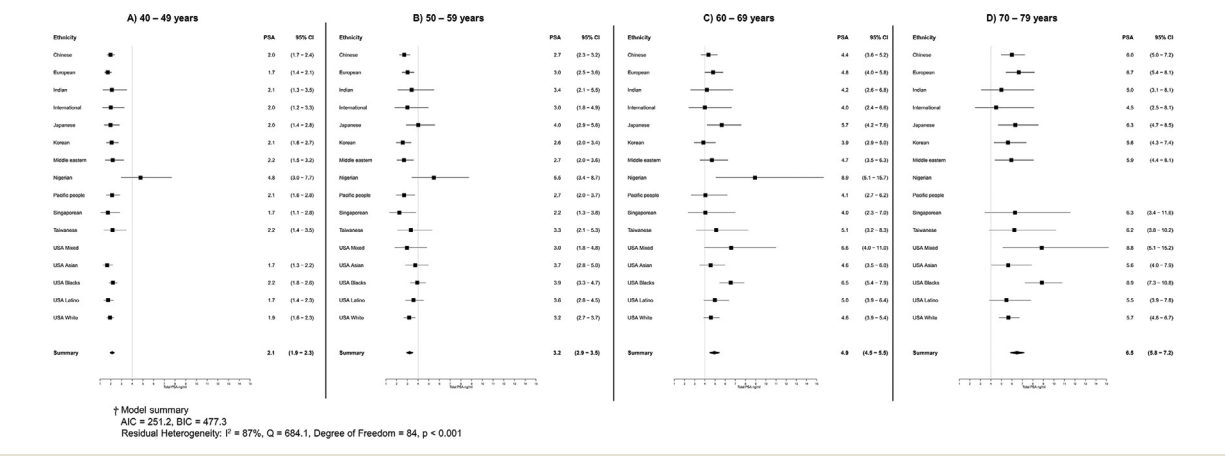
Author, Year	Ethnicity Reported	Ethnicity Grouped[b]	Age Group (Years)[a] 40-49		50-59		60-69		70-79	
			40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79
Sarma, 2014	USA White	USA White	1.8		2.9		4.6		7.1	
	USA Black	USA Black	2.4		3.3		6.1		5.6	
Ikuerowo, 2016	Nigerian	Nigerian	4.78		5.47		8.93		N/A	N/A
Yang, 2017	Chinese	Chinese	N/A	N/A	3.59		4.93		6.83	
Nan, 2018	Chinese	Chinese	1.92		2.63		4.41		6.2	

[a] N/A = not applicable
[b] Grouped ethnicity used in meta-analysis and forest plots

Table 3 Univariable Mixed-Effect Regression Analysis of Possible Contributors to Between Studies Heterogeneity in Age-Adjusted PSA Reference Values

Moderator/variable		AIC	Residual heterogeneity (%)	*P*
Study settings	Year (1993-2018)	13458.3	98.9	*.383*
	Prospective vs. retrospective	13462.1	99.1	*.427*
	Single vs. Multiple centres	13462.9	99.2	*.658*
	Objective to develop reference values (yes vs. no)	13461.9	99.2	*.246*
PSA assay type		13463.1	99.0	*.670*
Outcome measure	Observed vs. estimated	13463.1	99.2	*.676*
	Numerical vs. graphical illustration	13463.1	99.2	*.433*
Methodological quality		13461.3	99.1	*.239*
Ethnicity	Reported	13455.3	98.5	*<.001*
	Grouped	13458.9	98.6	*.008*
Age intervals	5- and 10-y	632.4	96.7	*<.001*
	10-y	651.1	96.7	*<.001*

Figure 2 Forest plots of age-adjusted upper PSA reference value grouped by ethnicity in multivariable mixed-effect regression meta-analysis. (A) participants aged 40-49 y, (B) participants aged 50-59 y, (C) participants aged 60-69 y, and (D) participants aged 70-79 y.



heterogeneity (Q = 684, degree of freedom = 84, $P < .001$). For men in their 40s and 50s, almost all ethnic groups had pooled upper total PSA limits <= 4.0 ng/ml. The only exception were Nigerian men. For men in their 60s and 70s, the lowest PSA values were observed in Korean and International ethnic groups, respectively. Conversely, the greatest values for the same age groups were observed in Nigerian and USA mixed men, respectively. However, the latter ethnic groups had the broadest observed 95% CI.

Discussion

To our knowledge, this is the first systematic review and meta-analysis of PSA reference values. Clear differences were observed in the upper PSA limits between the investigated studies. Methodological factors such as PSA assays, study quality, and statistical analyses, were not found to be significant drivers of the observed heterogeneity. On the other hand, age and ethnicity were independently associated with significant contribution to the differences in PSA reference values among the reviewed studies.

Oesterling and colleagues introduced the concept of age-adjusted reference values, in response to the observation of an increase in PSA levels with advancing age.[4] In that landmark study of Caucasian American men from the Olmsted country, the authors used the 95th percentile value as the upper limit of normal for PSA, within each age group. Conversely, Dalkin et al suggested that mean plus two Sd should be used instead of the 95th percentile.[3] However, since PSA is not normally distributed, the latter analysis had rapidly diminished and the 95th percentile method is currently the most acceptable in establishing upper reference limit for PSA.[12,30,44] The lower limit has frequently been described as the lowest detected by the assay analyser or the 5th percentile value, and often not reported due to the lack of clinical relevance.

The utility of the age-adjusted reference values of PSA in clinical practices has been controversial.[2,6] Numerous clinicians advocated the utilization of these values as upper limits, beyond which a prostate biopsy should be considered.[10,13,38,42,59] A report from Austria concluded that these limits could detect 8% more clinically important Pca in men aged 40 to 59 years and reduced the biopsy burden in older men by 21%.[59] These findings were supported by other studies from the United States demonstrating that age-adjusted reference values improved the predictive accuracy of PSA for Pca, when compared to a single cut-off value of 4.0 ng/ml.[13,60] However, this concept was criticized by several other investigators.[5,9,28] Catalona et al demonstrated that using the age-adjusted reference values, as opposed to a single cut-off of 4.0 ng/ml, had caused significant increase of unnecessary negative biopsies in younger men, and would have missed potentially curable cancers in men older than 60 years.[5] Similarly, Gustafsson and colleagues reported that higher PSA cut-offs for men in the latter age category, could miss several clinically significant cancers, that otherwise would have been detected with the 4.0 ng/ml cut-off.[28]

Despite the controversy, the development of age-adjusted reference values has brought the attention towards the presence of PSA variabilities among different populations.[10-12,33,41] Moreover, as illustrated in our review, it facilitated the detection of PSA differences among ethnic groups within the same population.[12-14,54] These variabilities were frequently attributed to differences in prostate volumes between the investigated cohorts. However, studies accounting for prostate size variations continued to demonstrate significant ethnic differences.[61-63] These findings are supported by our meta-analysis, since ethnicity was an independent contributor to the heterogeneity in PSA reference values.

The aetiology behind the ethnic variabilities in PSA levels remains poorly understood. In addition to differences in prostate sizes, the presence of subclinical Pca might have contributed to the heterogeneity. All the studies considered in this review had used certain set of criteria to assess men at risk of harbouring Pca. Having a PSA level above 4.0 ng /ml was the most frequent indication for warranting prostate biopsy. Catalona et al. reported the risk of detecting Pca in men aged 50 years or older with PSA between 2.6 and 4.0 ng/ml, as 22%.[64] Similarly, results from the Prostate Cancer Prevention Trial had demonstrated that 21.9% of the participants whom PSA levels were lower than 4.0 ng/ml, were found to have Pca on biopsy.[65] Nevertheless, the presence of ethnic differences in Pca risk among men with PSA less than 4.0 ng/ml is currently unknown. Further research using advanced diagnostic modalities such as multiparametric MRI[66], is required to investigate this aspect.

Another plausible explanation to the observed ethnic differences in PSA levels is the presence of biological variability at the cellular and molecular levels. Studies investigating prostate samples from men with Benign Prostatic Hypertrophy had demonstrated different cellular composition between Caucasian American, African American, Chinese, and Japanese men.[67,68] Additionally, several researchers have reported ethnic differences in androgens activity particularly between African American and Caucasian American men.[69] For instance, the less frequent occurrence of the CAG repeats in the androgen receptors of the former group, leading to increased activity of the receptors and greater responsiveness to a given level of androgen. However, the definitive correlation between these biological observations and PSA levels is yet to be quantified.

In addition to age and ethnicity, we assessed the impact of several methodological factors on the variabilities in PSA reference values (supplementary material Appendix D). Despite the lack of statistical significance (Table 3), these analyses provided information with clinical importance. Since the discovery of serum PSA, it became apparent that levels derived from different assays using the same sample would yield variable results.[58,70,71] Factors such as sample handling, analyzer's platform, assay kit, and calibre preparations have all been described as underlying precipitators for this phenomenon. The included articles in this meta-analysis were very inconsistent in describing this area of the methodological sections. Therefore, it was only possible to consider the assay analyzer's platform in the review. In our pooled data, there were no statistically significant differences observed in age-adjusted reference values derived from the different platforms. This might be due to the confounding effect of the other assay related aspects, which might have widened the confidence limits. Otherwise, the observed differences varied from 2% to 21%, which was comparable to previous reports addressing this topic at an individual level.[70,71] Thus, age-adjusted PSA reference values derived with a certain assay technique, might have limited generalisability in clinical practice adopting a different analyzer.

The results of this systematic review and meta-analysis need to be interpreted with consideration to three limitations. Firstly, we only included studies that had reported the 95th percentile values for PSA in the analysis. Theoretically, it is possible to estimate this value from the median or mean PSA level with Sd, using a complex extension of the formulas we adopted to derive the effect variance (supplementary material Appendix A). However, using these calculations would defer this study from its' aim to assess variability in age-adjusted reference values to differences in any PSA values. Secondly, majority of the included studies were not deemed to assume high methodological quality. Lastly, despite addressing several relevant factors, the residual heterogeneity between the studies remained significantly high ($I^2 = 87\%$). This suggests that other confounders beyond age and ethnicity, might have contributed to the observed differences in the PSA reference values.

Conclusions

It is evident that PSA levels increase with advancing age and significant differences exist in age-adjusted PSA reference values among the variable populations. Moderate quality data suggests that this could be partially explained by ethnic differences in PSA values obtained from apparently healthy men. Therefore, ethnicity is an important parameter that needs to be accounted for when interpreting PSA results in clinical practices.

Disclosure

The authors declare no conflict of interest and certify that they have no affiliations or involvement in any organization or entity with interest in the materials discussed in this manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.clgc.2021.11.014.

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