Clin Chem Lab Med 2012;50(5):879–884 © 2012 by Walter de Gruyter • Berlin • Boston. DOI 10.1515/CCLM.2011.776

Are the currently used reference intervals for creatine kinase (CK) reflecting the general population? The Tromsø Study

Hallvard Lilleng[1,2,*], Stein Harald Johnsen[1,2], Tom Wilsgaard[3] and Svein Ivar Bekkelund[1,2]

[1] Department of Neurology and Neurophysiology, The University Hospital of North Norway, Tromsø, Norway
[2] Institute of Clinical Medicine, University of Tromsø, Tromsø, Norway
[3] Institute of Community Medicine, University of Tromsø, Tromsø, Norway

Abstract

Background: Laboratory reference intervals are not necessarily reflecting the range in the background population. This study compared creatine kinase (CK) reference intervals calculated from a large sample from a Norwegian population with those elaborated by the Nordic Reference Interval Project (NORIP). It also assessed the pattern of CK-normalization after standardized control analyses.
Methods: New upper reference limits (URL) CK values were calculated after exclusion of individuals with risk of hyperCKemia and including individuals with incidentally detected hyperCKemia after they had completed a standardized control analysis. After exclusion of 5924 individuals with possible causes of hyperCKemia, CK samples were analyzed in 6904 individuals participating in the 6th survey of The Tromsø Study. URL was defined as the 97.5 percentile.
Results: New URL in women was 207 U/L. In men <50 years it was 395 U/L and in men ≥50 years 340 U/L. In individuals with elevated CK, normalization grade after control analysis was inversely correlated to the CK level ($p < 0.04$).
Conclusions: URL CK values in women and in men <50 years of age were in accordance with URL CK values given by the NORIP. In men ≥50 years, a higher URL was found and the findings suggest an upward adjustment of URL in this age group.

Keywords: creatine kinase (CK); epidemiology; hyperCKemia; population; reference values.

*Corresponding author: Hallvard Lilleng, Department of Neurology and Neurophysiology, The University Hospital of North Norway, 9038 Tromsø, Norway
Phone: +47 77 62 60 00, Fax: +47 77 62 70 74,
E-mail: hallvard.lilleng@unn.no
Received September 15, 2011; accepted October 13, 2011; previously published online November10, 2011

Introduction

Creatine kinase (CK) levels vary in different ethnicities; Black men and South Asians usually have higher CK values at rest than Caucasians (1–3). Epidemiological data from mixed racial populations has shown that inherent biological variation in CK values is probably wider than reflected in hospital reference intervals (4, 5). High CK values are a common finding in the general population, supporting the view that defining a cut-off for normality should be performed in large general populations (2–4, 6).

Usually, reference individuals are provided from laboratory staff, their acquaintances and from blood donors (7). However, by selecting "perfectly" healthy individuals with no evidence of disease as reference individuals, the reference intervals may not be concurrent with the normal range in the background population (8).

We have previously reported that 5.3% (9) from a Norwegian population had incidentally detected CK values beyond the upper reference limits (URL) elaborated by the Nordic Reference Interval Project (NORIP) (10). In subsequent standardized control analysis, about 70% normalized, giving a prevalence of persisting hyperCKemia of 1.3% and illustrating the great variability of this biological marker (9).

In the present report we calculated new URL CK values in the same population including data from individuals that completed standardized CK control analysis and excluding individuals with potential causes of hyperCKemia and compared them with URL values elaborated by NORIP (10, 11).

Special attention was paid to whether normalization of elevated CK occurred independently of CK level.

Materials and methods

Selection of reference individuals

We used an a posteriori sampling method, according to the Clinical and Laboratory Standards Institute C28-A3 document (12).

The reference individuals were recruited from the 6th survey of The Tromsø Study from October 2007 to December 2008 (13). The methods and the ethnic composition of The Tromsø Study are described elsewhere (9, 13, 14).

In brief, a questionnaire on demographics, education level, general health, familiar illnesses, muscle pain, psychiatric illnesses and use of healthcare was enclosed with the letter of invitation. Diet, alcohol and smoking habits, use of medication and leisure physical activity were also questioned. All attendants underwent physical examinations which included measurement of height, weight, waist and hip circumference, blood pressure and collection of blood samples.

CK was analyzed in 12,828 participants aged 30–87 years (mean 58 years), 6834 women and 5994 men, 64.9% of those eligible.

A total of 5924 (46.2%) individuals with potential causes of hyperCKemia were excluded; statin users (n=1846), those with hypothyroidism (n=1093), those with current kidney disease (n=525), those with hard leisure physical exercise defined as high activity (sweating or out of breath) at least 1 h/week (n=1361) and those with high alcohol consumption defined as drinking alcohol at least two times/week (n=2774).

A number of 562 out of 686 individuals with incidentally elevated CK values according to the NORIP reference intervals met a standardized CK control analysis 3 days after refraining from use of alcohol, leisure physical activity, muscular training, physiotherapy, acupuncture or any muscular damage (9). Data from the standardized CK controls were included after first excluding 143 individuals owing to use of statins or those reporting hypothyroidism or kidney disease. Seventy-two individuals reported to have hard leisure physical exercise and 65 reported high alcohol consumption, these 137 were all included as reference individuals as the standardized CK control analysis adjusted for these conditions.

Baseline characteristics of the remaining 6904 reference individuals are presented in Table 1.

The study was approved by the Regional Ethical Committee for Research and the Norwegian Data Inspectorate. Written informed consent was given by all participants.

Assay

CK was analyzed by photometry using an enzymatic method (CK-NAC, Roche Diagnostics®, Mannheim, Germany) at the University Hospital of North Norway in Tromsø. The samples were consecutively analyzed in an automated clinical chemistry analyzer (Modular P, Roche) within 6 h from withdrawal. The analytical coefficient of variation (CV_a) was ≤1.6% and the reference interval (2.5–97.5 percentile) for this method was that elaborated by the Nordic Reference Interval Project (NORIP); women aged ≥18 years, 35–210 U/L, men aged 18–50 years, 50–400 U/L and men ≥50 years, 40–280 U/L (10). When the CK level in a sample was beyond the upper limit, the sample was diluted according to recommendations given by the manufacturer.

The laboratory participates in LABQUALITY, a quality control system including about 100 laboratories, mainly in the Nordic countries. The Department of Laboratory Medicine receives control samples four times annually and has always had results within the analytical target limits of CK.

Table 1 Baseline characteristics in 6904 reference individuals.

	Men (n=3167)	Women (n=3737)
Age, years	55.8 (12.4)	56.2 (13.4)
Weight, kg	85.6 (13.5)	70.9 (13.2)
Height, cm	177.1 (6.9)	163.4 (6.5)
Body mass index, kg/m^2	27.3 (3.8)	26.6 (4.7)
Waist circumference, cm	99.3 (10.5)	90.8 (12.3)
CK, U/L[a]	120 (49–175)	86 (37–118)
S-creatinine, µmol/L	77.4 (13.4)	61.7 (11.0)
S-glucose, mmol/L	5.29 (1.24)	5.07 (0.94)
Systolic blood pressure, mm Hg	136.9 (20.1)	132.1 (24.7)
Diastolic blood pressure, mm Hg	81.1 (10.1)	74.8 (10.2)
Coronary heart disease, n (%)	91 (2.9)	70 (1.9)
Hypertension[b], n (%)	890 (28.1)	929 (24.9)
Diabetes mellitus, n (%)	121 (3.8)	117 (3.1)

Values are unadjusted mean (SD) or numbers (%). [a]Median (interquartile range, IQR). [b]Hypertension defined as systolic blood pressure ≥140 mm Hg and diastolic blood pressure ≥90 mm Hg or ever use of antihypertensive medication.

Statistical analysis

Inspection of the data in the reference sample group indicated a distribution skewed to the right and a significant kurtosis (range 10–7888 U/L, median 97; skewness 5.4 [SE 0.029], kurtosis 74.8 [SE 0.059]). The data did not fit a Gaussian distribution (Kolmogorov-Smirnov and Shapiro-Wilk tests of normality <0.001).

SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL, USA) and the SAS System version 9.2 (SAS Institute, Inc., Cary, NC, USA) were used for statistical analysis.

In order to reduce the skewness and kurtosis of the data, CK were raised to the power of –0.1 as the most optimal power transformation (using Box-Cox transformations). Fractional polynomials were fitted in a linear regression model to find the best relationship between the transformed CK and age. The powers for fractional polynomials were chosen from a set $\phi=(-2, -1, -1/2, 0, 1/2, 1, 2, 3)$ (15). The best fitting fractional polynomials was of degree 2 with powers –2 and 3 in men and –0.5 and 1 in women.

Age-related reference centiles were constructed using the absolute residuals approach proposed by Altman (16). Figure 1 shows back-transformed centiles to the original CK scale.

Visual inspection of the histograms revealed one man with a CK value 7888 U/L. After applying the Dixon range statistics ("one-third" rule) suggested by Reed et al. (17) this value was excluded as an outlier.

χ^2-test for cross tables was used to test differences between CK-quartiles and to test for linear trend in normalization after control analysis. A two-tailed p-value <0.05 was considered statistically significant.

Partition

The method introduced by Lahti et al. (18) was used in order to assess whether different reference limits should be reported for people aged <50 years and people aged ≥50 years. We identified sex-specific 2.5 and 97.5 reference limits for the combined data and used these limits to calculate the proportion of people who were above the 97.5 limit and the proportion of people who were below the 2.5 limit in each age group. The recommendation by Lahti et al. (18) is to partition the data if any of the four proportions is ≥4.1% or ≤0.9% and to combine the data if all proportions are between 1.8% and 3.2%. All other outcomes are considered as non-conclusive and the decision to partition or combine data should be based on clinical judgement and data from the literature (18).

In women, all proportions were between 1.8% and 3.2% and the data were combined. In men, the highest proportion was 3.3% and the lowest of the four was 1.4%, indicating no-conclusive results.

Figure 1 demonstrates the distribution of CK and reference centiles presented by age for both genders. Although visual inspection of the 97.5 percentile line in men did not indicate a clear cut-off at 50 years of age, we decided to report separate reference values in men <50 years and men ≥50 years. This in accordance with the NORIP Study (11), and was considered clinically reasonable.

Calculated reference limits

After deletion of individuals with potential causes of hyperCKemia and one outlier and including the data from individuals with completed standardized control CK analysis, we non-parametrically

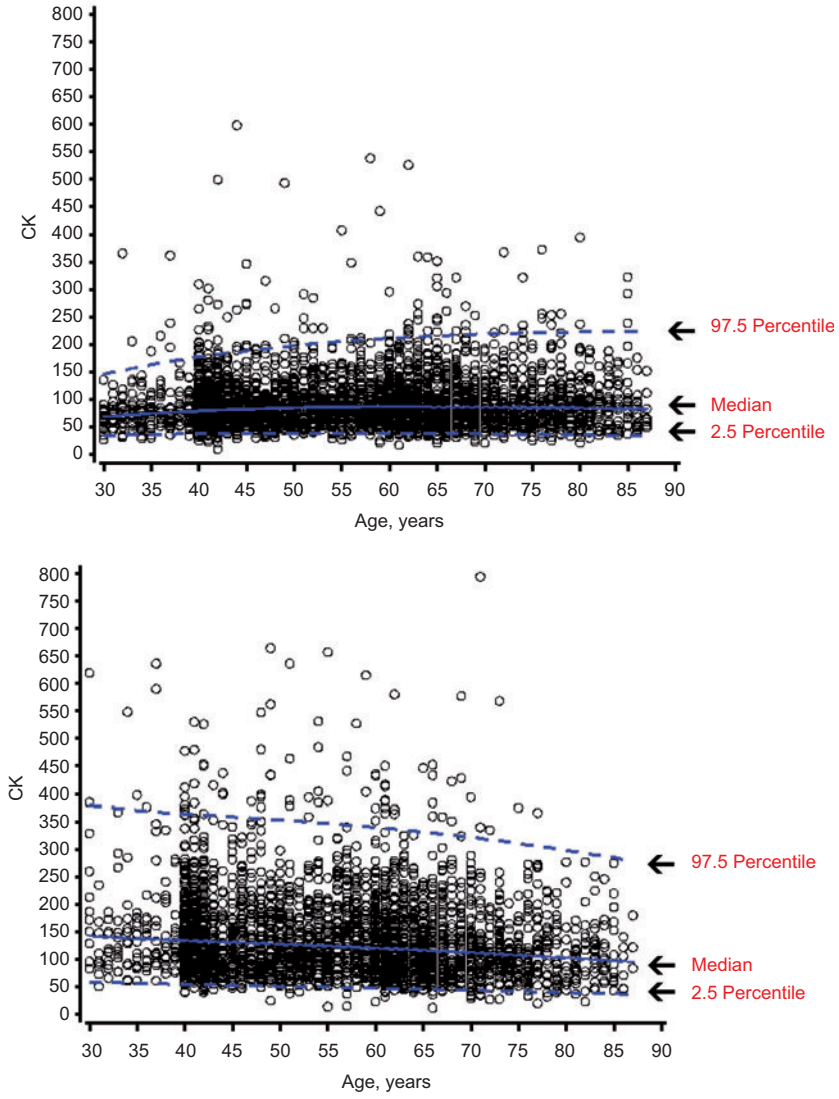

Figure 1 Distribution of CK in women (upper) and men (lower) by age (CK values >800 U/L are not shown).

calculated CK reference intervals according to the 2.5–97.5 percentile (12) and compared these with CK reference values elaborated by NORIP.

Results

In the reference sample population the 2.5–97.5 CK percentile interval (90% confidence interval, CI) in 3737 women was 37 (36–38) U/L–207 (197–212) U/L, compared to NORIP reference interval 35 (31–35) U/L–210 (180–233) U/L (10).

The corresponding finding in 1149 men <50 years of age was 57 (54–61) U/L–395 (362–416) U/L, compared to NORIP reference interval 50 (45–54) U/L–400 (351–487) U/L (10). In 2018 men ≥50 years the 2.5–97.5 CK percentile interval was 47 (44–51) U/L–340 (287–372) U/L, compared to NORIP reference interval 40 (36–46) U/L–280 (252–415) U/L (10).

In men, regardless of age, we calculated 2.5–97.5 CK percentile interval to 49 (48–52)–367 (341–384) U/L (Table 2).

The 2.5–97.5 percentiles in subgroups with potential causes of hyperCKemia, and in individuals which completed standardized control CK analysis are displayed in Table 2.

Normalization rate of elevated CK values after standardized control analysis (Figure 2) was highest in the lower CK quartile (76.4%) and declined in the upper quartile with a significantly linear trend ($p<0.04$).

Discussion

URL CK values in this Norwegian population were similar in women (207 vs. 210) and in men <50 years (395 vs. 400 U/L) compared to URL CK values given by the NORIP (10). In men ≥50 years, however, we found a significantly higher URL CK (90% CI) compared to NORIP; 340 (299–372) vs.

Table 2 URL CK values in reference individuals, in excluded subgroups with potential causes of hyperCKemia and in the group with completed stand. CK controls.

	n	2.5–97.5 Percentile CK U/L	Median CK U/L (range)
Reference individuals			
Women	3737	37–207	83 (10–1190)
Men	3056	49–367	121 (12–1860)
Statin users			
Women	848	38–266	94 (24–611)
Men	998	48–379	121 (20–1112)
Hypothyroidism			
Women	866	35–271	86 (18–582)
Men	227	46–433	126 (40–672)
Kidney diseases			
Women	290	36–289	84 (18–522)
Men	231	43–432	120 (22–2079)
Hard leisure physical exercise			
Women	618	39–268	90 (25–1088)
Men	743	57–525	141 (19–3029)
High alcohol consumption			
Women	1316	39–243	83 (19–716)
Men	1458	50–413	121 (20–3029)
Completed standard CK controls			
Women	249	52–449	172 (25–766)
Men	313	76–659	200 (46–1860)

280 (252–415) U/L. A strength of this study is that about five-times more men ≥50 years were included compared to the NORIP (2018 vs. 404 men) (10).

In a recent publication from our group, we found the prevalence of incidentally elevated CK to be 4.3% in women and 6.5% in men (9). After standardized control analyses, the prevalence of hyperCKemia based on NORIP references were reduced to 1.3% in women and 0.77% in men <50 years (9). In men ≥50 years, the hyperCKemia prevalence was 1.8% (9). Given a normalization rate of 70% after standardized control analysis in incidentally detected CK elevation (9), this may indicate that the URL NORIP values have been set too low in elderly men.

After exclusion of 46% of individuals with possible causes of hyperCKemia in the present study, the higher URL CK found in men ≥50 years compared to NORIP can hardly be explained by undetected neuromuscular disorders. Most important is probably biological variability. It is well accepted that CK needs to be standardized to be valid. If a standardized control was to be done in everyone above URL, we would expect 70% to normalize according to our previous findings (9). We informed all participants with incidentally detected hyperCKemia to refrain from physical exercise 3 days ahead of the control analysis. Three days was chosen for comparison with other studies and to minimise drop-outs, but may be too short an interval since the half-life of CK is considered to be 7 days or longer (19). The degree of normalization might therefore have been even higher than observed.

In addition, elderly people have a higher frequency of other somatic conditions, as well as a heavier burden of medication (polypharmacy), both which may influence on the CK level (20). Our population sample included individuals up to 87 years. The effect of age on CK in men from our data demonstrates the importance of including elderly people in such studies. To our knowledge, the effect of advanced age on the CK level has not been thoroughly investigated.

A possible bias to the result is the 124 (18%) individuals, 46 women and 78 men (53 men ≥50 years), that did not

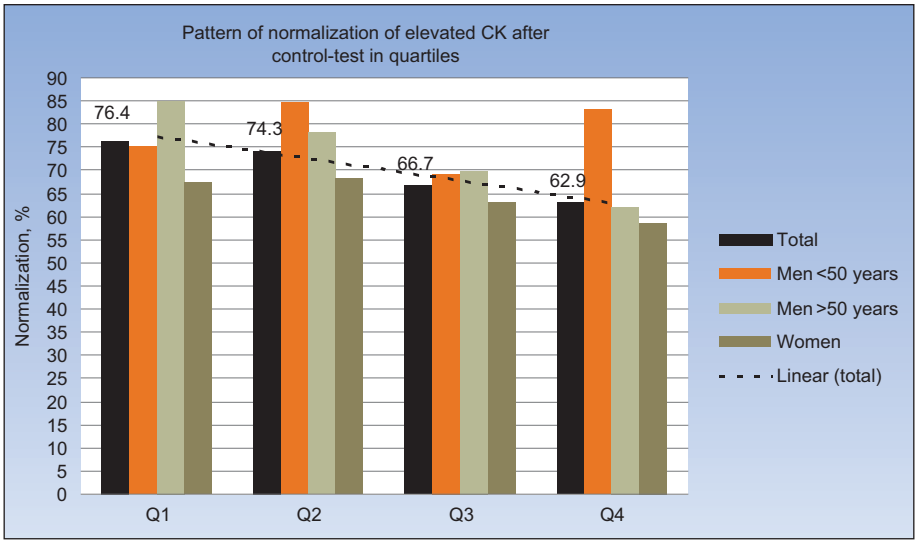

Figure 2 Pattern of normalization of elevated CK after control analysis in quartiles (Q1–Q4).
Q1: Women: 210–230 U/L, men ≥50 years: 280–312 U/L and men <50 years: 408–458 U/L. Q2: Women: 231–262 U/L, men ≥50 years: 315–358 U/L and men <50 years: 466–628 U/L. Q3: Women: 264–324 U/L, men ≥50 years: 361–448 U/L and men <50 years: 668–1028 U/L. Q4: Women: 327–3046 U/L, men ≥50 years: 448–2452 U/L and men <50 years: 1050–15,941 U/L.

complete the control CK test (9). Of the 53 men above 50 years, 21 were in the age group 50–60 years, 26 were between 60 and 70 years and six were above 70 years. However, the median CK values in these 53 men were fairly consistent with the 263 men ≥50 years who completed the control test, 365 U/L compared to 361 U/L (9). Assuming that about 70% would normalize if they had completed the control test, the CK levels in the remaining 16 men could hardly explain the differences in URL in men above 50 years.

We have shown that the normalization rate of elevated CK values was inversely correlated to the CK levels (Figure 2). The pattern of normalization has to our knowledge not been presented in prior studies. This information is considered important both in clinical praxis and for scientific purposes. Whereas lower supernormal CK values tend to be the result of random measurement variation and of less clinical importance, higher CK values more likely reflect a biological cause. High CK values is a common finding in the normal population, and asymptomatic or false-positive CK values occur frequently (1, 2, 4, 6). Too low or imprecise reference values may falsely categorise patients as having hyperCKemia and may exclude patients from participation in clinical trials (4). For instance, in patients with suspected statin induced myopathi URL CK levels are often used to decide whether to continue or end the statin therapy (4).

The effect on neuromuscular diagnosis after raising the URL in men from 174 U/L to 322 U/L and from 140 U/L to 201 U/L in women was assessed by Nardin et al. (21) in 2009. Increasing the URL resulted in a false-negative CK of clinical significance in seven of 94 subjects. The authors stated that the clinical impact of the loss in sensitivity was small and reduces unnecessary referrals and invasive testing in patients with asymptomatic CK elevations (21).

Taken together, our findings suggest an upward adjustment for URL in men ≥50 years. An upward adjustment of the URL CK values would reduce the proportion with clinical insignificant elevated CK values, and thereby decrease the burden of false-positive values.

In the NORIP project reasonable age limits for partition were estimated by "qualified guessing" prior to exposure to the partitioning program Refval 4.0 (10). Unlike NORIP we could not demonstrate an URL cut-off or partition of the data in men at 50 years of age (Figure 1). A possible explanation is that the participation in men <50 years in our study was lower compared to men above 50 years, which may cause selection-bias. Younger men have larger muscle mass and thereby have a physiological cause of higher basal CK levels than elderly men.

An argument for no partition of the data is to have one single URL CK value in men to relate to in clinical praxis. In a recent study from the Netherlands looking at the distribution of CK in a large random population sample with standardisation of exercise, URL was calculated to 322 U/L in white men regardless of age (4). The corresponding URL CK value in women was 201 U/L (4).

Despite numerous reported causes of increased CK, a group of individuals with "idiopathic" hyperCKemia has been subject to investigation (22–26). In our previous report, we found a possible cause in 46.2% of individuals with persistent hyperCKemia, where statin use explained nearly half (9). A total of 1846 (14.4%) individuals reported use of statins, 848 (12.4%) women and 998 (16.5%) men. Also statin users had a high normalization frequency, 71.7% in men and 57.9% in women, compared to 75.0% and 65.4% in non-users (9). To provide better information on the impact statins could have on CK, a case-control study with standardized CK measurements is warranted. A further clinical follow-up of individuals with persistent hyperCKemia could also enlighten the problem of balancing false-positive and false-negative values and thereby to set the most clinically relevant URL.

We have limited information about the 35% non-responders, which may cause selection-bias. This is especially of concern for the youngest men, where the CK variation is greatest and the attendance lowest (9). Another limitation is the lack of information in individuals younger than 30 years.

In summary, URL CK values in women and in men <50 years of age in this Norwegian general population were in accordance with URL CK values given by the NORIP.

In men ≥50 years we found a higher URL CK value, which could hardly be explained by undetected neuromuscular diseases, but most likely is caused by biological variability. The normalization rate of hyperCKemia according to the NORIP criteria was inversely correlated to the CK levels. Our findings question the need for an upward adjustment of the upper CK reference limits in men ≥50 years of age. Further studies should pay special attention to higher age groups.

Acknowledgments

We wish to thank Mrs Anna-Kirsti Kvitnes and Mrs Trine Storjord at the National Neuromuscular Centre, University Hospital North Norway, for practical and technical support with the study.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

References

1. Meltzer HY. Factors affecting serum creatine phosphokinase levels in the general population: the role of race, activity and age. Clin Chim Acta 1971;33:165–72.
2. Wong ET, Cobb C, Umehara MK, Wolff GA, Haywood LJ, Greenberg T, et al. Heterogeneity of serum creatine kinase activity among racial and gender groups of the population. Am J Clin Pathol 1983;79:582–6.
3. Morandi L, Angelini C, Prelle A, Pini A, Grassi B, Bernardi G, et al. High plasma creatine kinase: review of the literature and proposal for a diagnostic algorithm. Neurol Sci 2006;27:303–11.
4. Brewster LM, Mairuhu G, Sturk A, van Montfrans GA. Distribution of creatine kinase in a general population: implications for statin therapy. Am Heart J 2007;154:655–61.

5. Fraser CG. Inherent biological variation and reference values. Clin Chem Lab Med 2004;42:758–64.
6. Brewster LM, Mairuhu G, Bindraban NR, Koopmans RP, Clark JF, van Montfrans GA. Creatine kinase activity is assosiated with blood pressure. Circulation 2006;114:2034–9.
7. Henny J, Petitclerc C, Fuentes-Arderiu X, Petersen PH, Queralto JM, Schiele F, et al. Need for revisiting the concept of reference values. Clin Chem Lab Med 2000;38:589–95.
8. Ritchie RF, Palomaki G. Selecting clinically relevant populations for reference intervals. Clin Chem Lab Med 2004;42: 702–9.
9. Lilleng H, Abeler K, Johnsen SH, Stensland E, Løseth S, Jorde R, et al. Variation of serum creatine kinase (CK) levels and prevalence of persistent hyperCKemia in a Norwegian normal population. The Tromsø Study. Neuromuscul Disord 2011;21:494–500.
10. Rustad P, Felding P, Franzson L, Kairisto V, Lathi A, Mårtensson A, et al. The Nordic Reference Interval Project 2000: recommended reference intervals for 25 common biochemical properties. Scand J Clin Lab Invest 2004;64:271–84.
11. Rustad P, Felding P, Lathi A. Proposal for guidelines to establish common biological reference intervals in large geographical areas for biochemical quantities measured frequently in serum and plasma. Clin Chem Lab Med 2004;42:783–91.
12. CLSI. Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline – third edition. CLSI document C28-A3. Wayne, PA: Clinical and Laboratory Standards Institute, 2008.
13. Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njølstad I. Cohort profile: The Tromsø Study. Int J Epidemiol [E-pub ahead of print], DOI 10.1093/ije/dyr049.
14. www.tromsoundersokelsen.no.
15. Royston P, Altman DG. Regression using fractional polynomials of continuous covariates: parsimonious parametric modelling. Appl Statist 1994;43:429–67.
16. Altman DG. Construction of age-related reference centiles using absolute residuals. Stat Med 1993;12:917–24.
17. Reed AH, Henry RJ, Mason WB. Influence of statistical method used on the resulting estimate of normal range. Clin Chem 1971;17:275–84.
18. Lahti A, Hyltoft Petersen P, Boyd JC, Fraser CG, Jørgensen N. Objective criteria for partitioning Gaussian-distributed reference values into subgroups. Clin Chem 2002;48:338–52.
19. Serrao FV, Foerster B, Spada S, Morales MM, Monteiro-Pedro V, Tannus A, et al. Functional changes of human quadriceps muscle injury by eccentric exercise. Braz J Med Biol Res 2003;36:781–6.
20. Dugué A, Bagheri H, Lapeyre-Mestre M, Tournamille JF, Sailler L, Dedieu G, et al. Detection and incidence of muscular adverse drug reactions: a prospective analysis from laboratory signals. Eur J Clin Pharmacol 2004;60:285–92.
21. Nardin RA, Zarrin AR, Horowitz GL, Tarulli AW. Effect of newly proposed CK reference limits on neuromuscular diagnosis. Muscle Nerve 2009;39:494–7.
22. Capasso M, De Angelis MV, Di Muzio A, Scarciolla O, Pace M, Stuppia L, et al. Familial idiopathic hyper-CK-emia: an underrecognized condition. Muscle Nerve 2006;33:760–5.
23. Simmons Z, Peterlin BL, Boyer PJ, Towfighi J. Muscle biopsy in the evaluation of patients with modestly elevated creatine kinase levels. Muscle Nerve 2003;27:242–4.
24. Dabby R, Sadeh M, Herman O, Berger E, Watemberg N, Hayek S, et al. Asymptomatic or minimally symptomatic hyperCKemia: histopathologic correlates. Isr Med Assoc J 2006;8:110–3.
25. Prelle A, Tancredi L, Sciacco M, Chiveri L, Comi GP, Battistel A, et al. Retrospective study of a large population of patients with asymptomatic or minimally symptomatic raised serum creatine kinase levels. J Neurol 2002;249:305–11.
26. D'Adda E, Sciacco M, Fruguglietti ME, Crugnola V, Lucchini V, Martinelli-Boneschi F, et al. Follow-up of a large population of asymptomatic/oligosymptomatic hyperckemic subjects. J Neurol 2006;253:1399–403.