Original Article

Rainer Haeckel*, Werner Wosniok, Antje Torge and Ralf Junker

Age- and sex-dependent reference intervals for uric acid estimated by the truncated minimum chi-square (TMC) approach, a new indirect method

<https://doi.org/10.1515/labmed-2019-0164>

Received September 27, 2019; accepted January 28, 2020; previously published online May 22, 2020

Abstract

Background: Although the concentration of uric acid in serum or plasma is known to depend on sex and age and is subject to diurnal variation, the influence of these covariates on the reference interval (RI) is often neglected. Consequently, the values in the literature vary considerably. Therefore, we reinvestigated the reference limits and their dependence on covariates.

Methods: A new indirect approach was applied which derives a continuous function between age and RIs avoiding the usual “jumps” between various age groups.

Results: It is confirmed that the uric acid concentration in women is lower than in men. The RIs increase with age, in women more than in men. Between 80 and 90 years of age, the upper RI limit (RL) approximately reaches the same level in both sexes. Because the uric acid concentration may indicate renal insufficiency, the concentrations of creatinine and cystatin C were also measured. Both measurands showed the same behaviour as uric acid. Therefore, the age and sex dependency should be considered if the uric acid concentration is used as an indicator for hyperuricaemia (e.g. caused by gout or other metabolic diseases). Furthermore, a diurnal variation was observed.

Conclusions: Due to the variations of various covariates (age, sex, daytime, analytical systems), it is recommended that each laboratory should estimate its own RIs.

Keywords: age and sex dependency; reference intervals; uric acid.

Introduction

Reference intervals (RIs) of uric acid still vary considerably in the literature due to different analytical procedures and different reference subpopulations and because biological variables influencing the RIs are not considered. These variables are mainly sex and age [1]. Direct and indirect approaches have been applied to determine RIs. Whereas the statistical procedure for direct methods is well accepted, several indirect models have been described which have not reached general consensus. On the other side, direct methods require considerable expenses and are less suited for stratification according to sex and age contrary to indirect methods [2–4].

Recently, we have described a new indirect approach (truncated minimum chi-square, TMC), which overcomes most of the disadvantages other indirect methods have [4]. This technique is now applied to reinvestigate the RI limits (RLs) of uric acid in serum or plasma. The measurement values for uric acid obtained from three routine laboratories are compared with each other and with reports in the literature. Furthermore, diurnal variation is studied.

Hyperuricaemia as a predictor for preeclampsia is not covered by the present study.

Materials and methods**TMC approach**

The TMC method was described recently in detail [4]. The method first identifies an interval containing essentially non-diseased patients, the truncation interval. Then, a power normal distribution (PND) is fitted to this interval by an iterative minimum chi-square approach. This approach accounts for the fact that only a part of all values from non-diseased patients is used for parameter estimation. Assuming a PND for values of non-diseased patients is an essential component of

*Correspondence: Rainer Haeckel, Institute for Laboratory Medicine, Katrepeler Landstr. 45E, 28357 Bremen, Germany, Phone: +49 412 273448, E-Mail: rainer.haeckel@gmx.net

Werner Wosniok: Institut für Statistik, Universität Bremen, Bremen, Germany

Antje Torge and Ralf Junker: Institut für Klinische Chemie, Universitätsklinikum Schleswig-Holstein, Kiel, Germany

the TMC approach. No assumption is made about the distribution of values from diseased patients. RLs are calculated from the estimated PND parameters. The prevalence of non-diseased patients in the data results from the PND and the estimated parameters: prevalence = (number of values in the truncation interval) / ([probability assigned to the truncation interval by the PND] [total number of values]).

The present study uses an enhanced version of the procedure described in [4]. Now, the size of the truncation interval is automatically determined on the basis of fit criteria. The truncation interval size was formerly fixed at 70% of all values and is now automatically selected from the range 60%–80%. The selection criteria for truncation intervals are: (i) the *p* value for the goodness of fit must be >0.05 and (ii) the estimated prevalence must be ≥0. If these conditions hold for several truncation interval candidates, the largest interval is used for parameter estimation. To ensure condition (ii), a corresponding penalty term was added to the estimation procedure as an additional constraint.

A script based on the R programming language with automatic partitioning for age and sex, and calculation of confidence limits can be requested from the authors. The analysis is automatically stratified by age groups and sex, if the data allow so. Age groups are defined by the user. For adults, 10-year intervals are used in the present study. Five-year interval led to the same spline function, but their confidence intervals were slightly larger, as expected. Age-specific results are combined by spline functions to generate continuous relations between the patients' age and RLs, separately for each sex. The spline function provides numerical RL estimates for all ages covered by the data. Their graphical presentation has no artificial "jumps" between different ages.

Furthermore, the programme automatically estimates the diurnal variation if the sampling times are given in the data. Instead of the sampling time, we used the time samples arrived in the laboratory assuming that the average difference between sampling and arrival time was approximately 2 h.

Differences between RLs were considered as relevant if the critical difference DC was surpassed. DC was estimated on the basis of the permissible imprecision as recently suggested [5]. It can be calculated by a software tool available free of charge on the home page of the DGKL [6].

Data selection and sources

The laboratory results of patients were selected as described earlier [7]: only first values were used if several

results from the same patient were obtained during a hospital stay or during the time when the data were selected. First values were chosen to avoid ties (correlated observations from the same patient, which is against the assumptions of both direct and indirect methods).

The new proposal was used with real data obtained by routine laboratories, either of the primary health care sector (private laboratories serving mainly general practitioners) or from a large university hospital (laboratory 3) as indicated in the legends of the figures. Laboratory 1 did not receive samples of hospitalised patients and laboratory 2 only from less than 5% of hospitalised patients. Laboratory 3 excluded hospitalised patients from special wards (e.g. intensive care units and gynaecological units to minimise the number of pregnant women in the data analysed). Subpopulations of the primary health care sector usually have lower prevalence (lower percentage of diseased subjects) than hospitalised patients. Therefore, no subjects were excluded from primary health care data, in contrast to data from hospitalised patients. The laboratories which provided the measurement values were accredited according to ISO 15189 and followed the Guidelines of the German Medical Association on Quality Assurance in Medical Laboratory Examinations (RiliBAEK) [8]. The analytical methods were applied as described by the manufacturers, the uric acid concentrations were determined with a uricase/peroxidase procedure in laboratory 1 with AU 5800 (Beckman Coulter, Krefeld, Germany), in laboratories 2 and 3 with Cobas 8.000 c/02 (Roche, Basel, Switzerland). The creatine concentrations were determined with an enzymatic procedure 2 and a kinetic modification of the Jaffé procedure in the other laboratories. The cystatin C was measured with a nephelometer BN2 (Siemens, Erlangen, Germany), an enzymatic procedure in laboratory 2 and a kinetic modification of the Jaffé procedure in the other laboratories.

The time for collecting the data (data acquisition time) was either one or several years. The stability of the analytical procedure during the data acquisition time was verified by plotting the monthly medians with confidence limits as described elsewhere [6, 7].

Results

Uric acid

The present study confirms that male subjects have higher RLs than women as also reported by others (see Table 1), and that the RLs increase with age in both genders

Table 1: TMC reference intervals (95% central interval) for the uric acid concentration in serum or plasma (μmol/L).

Gender	Age, years	Lab. 1, non-hospitalised	Lab. 2, non-hospitalised	Lab. 3, non-hospitalised	Lab. 3, hospitalised	DC interval ^a	Literature
Male	18–29	RI (n) ^b 239–521 (5530)	RI (n) 213–470 (21,302)	RI (n) 218–515 (3853)	RI (n) 210–533 (3392)	200–226/445–495	230–480 [9]
	40–49	238–544 (11,832)	229–507 (47,303)	222–558 (5229)	223–552 (5124)	215–243/480–534	200–568 [10]
	80–90	236–628 (20,784)	222–599 (18,412)	194–616 (2656)	209–693 (10,492)	206–238/563–635	160–450 [11] 214–488 [12]
Female	18–29	168–423 (15,439)	158–363 (24,827)	141–386 (4794)	143–434 (5260)	148–168/343–383	155–355 [9]
	40–49	169–424 (21,869)	155–379 (48,985)	154–434 (5909)	144–425 (5745)	144–166/357–401	150–472 [10]
	80–90	198–617 (23,012)	176–621 (27,172)	168–615 (2148)	158–758 (11,499)	161–191/580–662	110–330 [11] 137–363 [12]
		AU 5800 Beckman Coulter	Cobas 8000 c702 Roche Diagnostics	Cobas 8000 c702 Roche Diagnostics	Cobas 8000 c702 Roche Diagnostics		

^aInterval of the critical difference based on the RIs of laboratory No. 2. ^bReference interval (number of contributing values).

(Figure 1). The upper RIs (97.5th percentile) in women increased more distinctly and reached approximately the same level in the last decade studied as in men. With the TMC method, the upper RI concentration in serum or plasma determined in various laboratories varied in men between 470 and 693 μmol/L (Table 1) and in women between 363 and 758 μmol/L. Literature data varied between 473 and 568 μmol/L for men and between 330 and 472 μmol/L for women. The lower RIs were almost constant between 18 and 100 years of age.

In laboratory 2, male subjects (18–29 years) had a TMC upper RI of 470 μmol/L. Estimation of the permissible difference on the basis of the permissible analytical imprecision was calculated as recently proposed [13]. The permissible difference limits are 445–495 for the upper RI of 470. These limits are surpassed in three laboratories in the age interval 18–29 years (marked in red in Table 1). Therefore, common RIs of the age interval 18–29 years cannot be justified.

In laboratory 3, the data could be split into non-hospitalised patients (outpatients, ambulant patients) and into hospitalised patients. The upper RIs of both groups were similar up to the age of 60 years (Table 2). In the last decade studied (80–90 years), the upper RIs increased more dramatically in the hospitalised group than in the outpatient group. This unexpected phenomenon could have several reasons: (1) a shift of the whole distribution towards higher values, (2) a critical increase of the prevalence of pathological values which cannot be tolerated by the TMC approach or (3) a broader distribution range leading to higher standard deviations (SDs) and hence to higher upper RIs.

A shift can be excluded because the modes of both distributions (80–90 year subgroups of ambulant vs. hospitalised patients) were approximately the same (Table 2). A relatively high prevalence of “pathological values” could also be excluded (Table 2). However, the range of the data distribution and the SDs of the distributions in the older group were higher in hospitalised patients than in ambulant patients (SD = 115 vs. 156 with women, Table 2). One reason for a broader distribution pattern could be a lower mean glomerular filtration rate in the hospitalised group. Therefore, we prefer the last explanation as the most plausible one because the creatinine concentration was also higher in the older group of hospitalised patients (Table 3).

Furthermore, the data sets could be stratified according to the time when the samples arrived in the laboratory. If it is assumed that the transport times are approximately the same for all samples, a diurnal variation can be observed as has also been reported by others [14, 15]. This

Figure 1: Reference limits of serum uric acid ($\mu\text{mol/L}$) depending on age (years) separated by sex.

Lower lines represent the 2.5th percentile, upper lines the 97.5th percentile, blue lines represent men ($n = 24,582$) and red lines women ($n = 24,579$). The data were obtained from primary health care laboratories without exclusion of data (A: laboratory 1, $n = 339,192$;

B: laboratory 2, $n = 615,290$), and from a university hospital (a mixture of hospitalised patients and outpatients; C: laboratory 3, $n = 330,589$).

Table 2: Reference intervals of uric acid concentrations in heparinised plasma ($\mu\text{mol/L}$) with prevalence, mode, range and standard deviation (SD) of distribution, and number of contributing values (laboratory 3).

Ambulant patients		RIs	Prevalence, %	Mode	Range of RIs	SD	n
Male	18–29	218–515	16.7	230	297	76	3853
	80–90	194–616	17.2	360	422	108	2656
Female	18–29	141–386	15.9	230	205	63	4794
	80–90	168–615	16.8	289	447	115	2221
Hospitalised patients							
Male	18–29	210–533	11.1	315	323	83	3392
	80–90	209–693	7.8	346	484	125	10,492
Female	18–29	143–434	10.5	230	291	75	5260
	80–90	158–758	8.1	295	600	156	11,499

Table 3: Reference intervals ($\mu\text{mol/L}$) with range and number (n) of distributions of creatinine concentrations in heparinised plasma (laboratory 3).

Ambulant patients	Age	RLs	Range ^a	n
Male	18–29	65–104	39	16,652
	80–90	63–155	92	8883
Female	18–29	51–83	32	21,048
	80–90	51–116	65	8324
Hospitalised patients				
Male	18–29	59–106	47	16,483
	80–90	51–177	126	29,475
Female	18–29	43–84	41	21,066
	80–90	49–123	74	33,542

^aRange of reference intervals.

Figure 2: Diurnal variation of the uric acid concentration in heparinised plasma (ambulant patients, laboratory 3, $n=80,099$). Daily observations are calculated during 1 week and the medians from Monday to Friday are presented. The yellow areas represent the approximate 95% confidence limits based on the median absolute deviation.

variation has a similar pattern from Monday to Friday. If the values from Monday to Friday are combined (Figure 2, not stratified by sex and age), a maximum was detected at about 7:00 am and a minimum during the night. Sennels et al. [14] reported a peak at about 5:00 am (sampling time) which corresponds to our maximum at 7:00 am for the arrival time in the laboratory if we assume an average transport time of about 2 h. The diurnal variation appeared in ambulant patients, but not in hospitalised patients. This divergence cannot be explained.

Creatinine and cystatin C

RLs of men for the creatinine concentration in serum are higher than those of women confirming earlier reports (Figure 3). The limits are almost constant up to about 50 years in both genders, and then, they rise with increasing age. The gender difference is usually attributed to the lower muscle mass of women. The same pattern of age and sex dependency was observed in the other two laboratories. Cystatin C was determined in serum samples obtained from primary care patients. Age dependency was similar to creatinine (Figure 4). The upper RL was constant between the age of 18 and 50 years, and, thereafter, the concentration increases

Figure 3: Reference limits of serum creatinine ($\mu\text{mol/L}$) depending on age (years) separated by sex.

Lower lines represent the 2.5th percentile, upper lines the 97.5th percentile, blue lines represent men ($n=386,317$) and red lines women ($n=460,846$). The data were obtained from two primary health care laboratories (A: laboratory 1, $n=327,132$; B: laboratory 2, $n=847,163$).

Figure 4: Reference limits of serum cystatin C (mg/L) depending on age (years) separated by sex.

Lower lines represent the 2.5th percentile, upper lines the 97.5th percentile, red lines represent men ($n = 6131$) and blue lines women ($n = 5266$). The data were obtained from a primary health care laboratory (laboratory 2).

with progressing age. No significant differences exist between men and women, because the confidence limits overlap almost completely (Figure 4).

Discussion

The creatinine and cystatin C concentrations are well-known measurands indicating renal glomerular function. The blood concentrations of both analytes increase with age. This phenomenon is usually explained by a decrease of the glomerular filtration rate in older people. The SEN-IORLAB study was probably the first that revealed age- and sex-specific RIs of cystatin C indicating a relative retention of biologically active low-molecular-weight compounds [16]. The age dependency of creatinine and cystatin C concentrations (Figures 3 and 4) looks very similar to that observed with uric acid rising above the age of 50 years. Therefore, it can be assumed that the rise of the uric acid concentration above the age of 50 years may be caused by the decrease of the glomerular filtration rate.

The age and sex dependency is very similar with all three measurands studied and should be considered for diagnostic purposes. Furthermore, the upper RIs of uric acid differed in the three laboratories studied even if the same analytical system was applied. Some differences surpassed the critical difference justifying the recommendation that each laboratory should estimate its own RIs.

An official German guideline [17] states that the uric acid concentration in serum is below the upper RI in one third of patients with an acute gout attack. This statement may not be correct in the light of the age dependency found here. Even the lowest upper RI value is above the physicochemically defined solubility of uric acid at $\text{pH} = 7.4$ and 37°C ($=387\ \mu\text{mol/L}$). Apparently, this solubility value cannot be applied for the probability of gout occurring because it would lead to unrealistic high rates of this disease (high rates of false-positive results). Whether the RIs estimated by the present approach can be used for the diagnosis of hyperuricaemia (e.g. caused by gout) depends on the rate of false-positive results, respectively of false-negative results which must be clarified by clinical studies.

Usually, diurnal variation is determined from multiple measurements per individual. This approach is relatively expensive and time-consuming and, therefore, the contributing number of patients is limited. With the TMC programme, single measurements from many individuals are used, and, thereby much larger numbers can be included. The uric acid concentration gradually increased through the night with peaking about midnight and a minimum at about 7:00 am (Figure 2) assuming that the average transport time of the samples might have been about 2 h. The difference between the maximum and minimum concentrations was about $120\ \mu\text{mol/L}$. The diurnal variation could explain that the upper RIs were lower in non-hospitalised patients than in hospitalised patients. The samples may usually be taken during the late morning in ambulatory patients whereas the samples taken in hospitalised patients are usually taken during the whole day.

Seasonal differences of about 17% were observed [15]. The authors considered the observed differences of no diagnostic importance in practice.

In conclusion, the uric acid values were higher in laboratory 1 (Beckman AU) than in the other laboratories (using analytical systems from Roche). In the laboratories using the Roche analysers, the upper RIs were slightly higher in hospitalised patients than in ambulatory patients. Therefore, RIs should be established for each laboratory. They should be stratified according to sex and age. Diurnal variation must also be considered with ambulant patients. Laboratories not being able to determine their own RIs may cooperate with other laboratories applying the same analytical systems and serving comparable subpopulations in order to derive common RIs.

Acknowledgments: The authors are grateful to Dr. Alexander Bertram (laboratory 1, private laboratory), Dr. Alexander Krebs (laboratory 2, private laboratory) and Dr. Antje Torge (laboratory 3, university hospital), for providing “big data” pools of patients.

Research funding: None declared.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

References

- Özcürümez M, Haeckel R. Biological variables influencing the estimation of reference limits. *Scand J Clin Invest Lab Med* 2018;78:337–45.
- Haeckel R, Wosniok W, Arzideh F. A plea for intra-laboratory decision limits. Part 1 general consideration and concepts for determining decision limits. *Clin Chem Lab Med* 2007;45:1033–42.
- Jones GR, Haeckel R, Loh TP, Sikaris K, Streichert Th, Katayev A, et al. Indirect methods for reference interval determination – review and recommendations. *Clin Chem Lab Med* 2019;57:20–9.
- Wosniok W, Haeckel R. Indirect estimation of reference intervals by a truncated penalised minimum chi-square approach. *Clin Chem Lab Med* 2019;57:1933–47.
- Haeckel R, Wosniok W, Arzideh F. Equivalence limits of reference limits for partitioning of population data. Relevant differences of reference intervals. *J Lab Med* 2016;40:199–205.
- German Society of Clinical Chemistry and Laboratory Medicine. Decision limits/guideline values. www.dgkl.de/arbeitsgruppen/entscheidungsgrenzen-richtwerte (accessed 18.12.18).
- Arzideh F, Wosniok W, Gurr E, Hinsch W, Schumann G, Weinstock N, et al. A plea for intra-laboratory reference limits. Part 2. A bimodal retrospective concept for determining reference intervals from intra-laboratory data bases demonstrated by catalytic activity concentrations of enzymes. *Clin Chem Lab Med* 2007;45:1043–57.
- Richtlinie der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen (Rili-BÄK). *Dtsch Arztebl* 2014;38:A1583–618.
- Rustad P, Felding P, Franzson L, Kairisto V, Lahti A, Martensson 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.
- Kallner A, Gustavsson E, Hendig E. Can age and sex related reference intervals be derived for non-healthy and non-diseased individuals from results of measurement in primary health care? *Clin Chem Lab Med* 2000;38:633–54.
- Ozarda Y, Aslan D. Use of total patient data for indirect estimation of reference intervals for 40 clinical chemical analytes in Turkey. *Clin Chem Lab Med* 2006;44:867–76.
- Gresser U, Gathof B, Zöllner N. Uric acid levels in southern Germany in 1989. *Klein Wochenschr* 1990;68:1222–8.
- Haeckel R, Haeckel R, Wosniok W, Gurr E, Peil B. Permissible limits for uncertainty of measurement in laboratory medicine. *Clin Chem Lab Med* 2015;53:1161–71.
- Sennels HP, Jorgensen HL, Goewtze JP, Fahrenkrug J. Rhythmic 24-hour variations of frequently used clinical biochemical parameters in healthy young males – the Bispebjerg study of diurnal variations. *Scand J Clin Lab Invest* 2012;72:287–95.
- Touitou Y, Touitou C, Bogdan A, Reinberg A, Motohashi Y, Auzepy A, et al. Circadian and seasonal variations of electrolytes in aging humans. *Clin Chim Acta* 1989;180:245–54.
- Risch M, Sakem B, Risch L, Nydegger UE. The SENIORLAB study in the quest for healthy elderly patients. *J Lab Med* 2018;42:109–20.
- Kiltz U. S2e-Leitlinie Gichtarthritis. Evidenzbasierte Leitlinie der Deutschen Gesellschaft. für Rheumatologie. AMMF Leitlinien Register Nr.060/005.