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

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Original Article

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...
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 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
Haeckel et al.: Age- and sex-dependent RIs for uric acid estimated by the TMC approach

(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 RL 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 RLs were almost constant between 18 and 100 years of age.

In laboratory 2, male subjects (18–29 years) had a TMC upper RL 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 RL 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 RLs of both groups were similar up to the age of 60 years (Table 2). In the last decade studied (80–90 years), the upper RLs 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 RLs.

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 compared to the ambulant 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 variation was also observed in Table 3. The diurnal variation showed that the upper RLs were higher in the morning than in the afternoon.

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

| Gender | Lab. 1, non-hospitalised | Lab. 2, non-hospitalised | Lab. 3, non-hospitalised | Lab. 3, hospitalised | DC interval | Literature |
|--------|------------------------|------------------------|------------------------|---------------------|-------------|------------|
| Male   | Age, years             | RI (n)                 | RI (n)                 | RI (n)              | RI (n)      | Literature |
| 18–29  | 239–521 (5530)         | 213–470 (21,302)       | 218–515 (3853)         | 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]|
| Female | Age, years             | RI (n)                 | RI (n)                 | RI (n)              | RI (n)      | Literature |
| 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)      | 144–166/375–401 | 150–672 [10]|
| 80–90  | 198–617 (23,012)       | 176–621 (27,172)       | 168–615 (2146)         | 158–758 (11,499)    | 161–191/580–662 | 110–330 [11]|
|        | AU 5800                | Beckman Coulter        | Roche Diagnostics      | Roche Diagnostics   | Roche Diagnostics |

*Interval of the critical difference based on the RLs of laboratory No. 2. †Reference interval (number of contributing values).
Figure 1: Reference limits of serum uric acid (μmol/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 (μmol/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 | RIs      | Prevalence, % | Mode | Range of RIs | SD  | n    |
|-----------------------|----------|---------------|------|--------------|-----|------|
| 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|
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.

### Table 3: Reference intervals (μmol/L) with range and number (n) of distributions of creatinine concentrations in heparinised plasma (laboratory 3).

| Ambulant patients | Age | RLs    | Rangea | 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.

### Figure 3: Reference limits of serum creatinine (μmol/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).
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 SENIORLAB 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 RLS 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 RL 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 RL value is above the physicochemically defined solubility of uric acid at pH = 7.4 and 37 °C (=387 μmol/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 μmol/L. The diurnal variation could explain that the upper RLS 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 RLS 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.

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