Reference intervals and decision limits

W213

EXPERIENCE OF CLINICAL LABORATORY IN ESTABLISHING OF REFERENCE INTERVALS FOR ACCESS FREE T4 AND ACCESS FREE T3 ASSAYS TESTS RESULTS, SPECIFIC FOR ITS OWN POPULATION USING INDIRECT SAMPLING TECHNIQUE

N. Bondareva [3], N. Kotelnikova [3], B. Schloeder [1], A. Ruzhanskaya [2]
[1]*Beckman Coulter Inc., Chaska, USA*
[2]*Beckman Coulter LLC, Moscow, Russia*
[3]*Regional Clinical Consultative and Diagnostic Center, Stavropol, Russia*

BACKGROUND-AIM

Each clinical laboratory is expected to establish its own reference intervals (RIs) as recommended in the IFCC/CLSI guideline (C28-A3) [1]. Due to difficulties in applying of direct approach for RIs calculation, most laboratories in Russia use RIs provided by the reagent manufacturers, but they may not match to the Russian population. RIs, specific for patient population of laboratory, were calculated for Access Free T4 and Access FT3 assays using easy to use indirect method and compared to RIs were established during Russian part of IFCC Committee on Reference Intervals and Decision Limits (C-RIDL) project [2].

METHODS

Based on [1], inclusion criteria were defined as presence of TSH result inside of respective RI is using in laboratory for all FT4 and/or FT3 results (n>600 each) were included in the retrospective study. 16,172 patient sample results were tested for TSH, Free T4, and/or Free T3 and were determined to have normal TSH values as defined by a TSH reference interval of 0.40 – 4.00 μIU/mL. 95%, 97.5%, and 99% reference intervals were #alculated for Access Free T4 and Access Free T3 based on the distribution of results defined as TSH normal. For calculation EP Evaluator software was used.

RESULTS

RIs for Free T4 (ng/dL) were defined as following: 95% 0.49 – 1.10 (IFU: 0,61-1,12; IFCC C-RIDL project: 0.65 – 1.10); 97.5% 0.47 – 1.17; 99% 0.43 – 1.25. Upper limit (UL) of newly calculated 95% RI was close to those provided in manufacturers' IFU and established in IFCC C-RIDL project for Russian population using direct approach [2]; low limit (LL) was significantly lower (r<0.001).
RIs for Free T3 (pg/mL) were defined as following: 95% 2.61 – 4.43 (IFU: 2,5-3,9; IFCC C-RIDL project: males: 2.83-4; females: 2.69-3.96); 97.5% 2.46 – 4.69; 99% 2.36 – 5.31. Newly calculated FT3 UL was statistically higher in comparison to those provided in IFU and [2], as opposite to LLs, which was comparable.

CONCLUSIONS

Current approach can aid laboratories in establishing the most appropriate population-specific Free T4 and Free T3 reference intervals and could be easily implemented in routine practice. CLSI Guideline EP28-A3c provides additional guidance for laboratories on how to properly verify new reference intervals prior to implementation.

Reference intervals and decision limits

W214

NEUROFILAMENT LIGHT CHAIN CONCENTRATIONS IN SERUM OF AGING POPULATION

A. Ladang [1], S. Kovacs [1], L. Lengelé [2], M. Locquet [2], J. Reginster [2], O. Bruyère [2], E. Cavalier [1]

[1]*Clinical chemistry department, CHU de Liège, Liège*

[2]*WHO Collaborating Centre for Public Health Aspects of Musculoskeletal Health and Aging, Division of Public Health, Epidemiology and Health Economics, University of Liège, Belgium*

BACKGROUND-AIM

Neurology blood-based biomarkers are now emerging at an impressive speed. Among them, neurofilament light chains (NF-L) are one of the most promising and studied blood-based neurological biomarkers. Indeed, NF-L expression is now recognized to be modified in a broad spectrum of neurological diseases and traumatic brain injuries. Because many neurological diseases are also aging diseases, it is now urgent to understand influencing factors of NF-L expression in normal aging population.

METHODS

In this study, we are assessing NF-L concentration in sera of a large cohort of 409 community-dwelling adults aged over 65 years old. We studied the link between NF-L and various physiological factors (age, sex, BMI, GFR,…) but also with self-reported comorbidities or life-style habits.

RESULTS

We showed that NF-L concentrations in serum are tightly linked to cystatin C concentrations ($r=0.5007$, $p<0.0001$) and consequently to estimated glomerular filtration rate and renal function ($r=-0.492$; $p<0.0001$). We also observed that NF-L expression is dependent on age and BMI but not sex in both univariate and multivariate analysis. Among the self-reported comorbidities, subjects having reported neurological disorders, cardiovascular diseases or history of fracture had higher NF-L concentrations in univariate analysis. In the multivariate model, only subjects with self-reported neurological disorders showed higher on NF-L concentrations. NF-L concentrations were also increased when mini-mental state examination (MMSE) was decreased below 26 points but not when geriatric depression score (GDS) was increased above 5 points in both univariate and multivariate analysis. According to these results, we established reference ranges by age categories for subjects with or without altered renal function and we showed that renal status has more impact on reference ranges than age.

CONCLUSIONS

Our data highlight that NF-L concentrations in aging population are not driven by increasing number of comorbidities or depression. Yet, as many other blood biomarkers, NF-L blood concentrations are dependent on a proper kidney function and NF-L interpretation in patients suffering from renal failure should be taken with caution.

Reference intervals and decision limits

W215

SERUM REFERENCE INTERVALS OF ZINC AND COPPER IN HEALTHY SUBJECTS OF TURKEY POPULATION

M. Demirel [1], S. Selek [1], T. Yildiz [1], R. Sonmez [2], A.Z. Gul [1], U. Sarikaya [1], F. Koktasoglu [1]
[1]*Department of Medical Biochemistry, Faculty of Medicine, Bezmialem Vakif University, Istanbul/Turkey*
[2]*Faculty of Medicine, Bezmialem Vakif University, Istanbul/Turkey*

BACKGROUND-AIM

Copper and zinc both play a significant role in the proper function of female sex hormones. We aimed to determine the reference intervals of serum zinc and copper in the healthy population of Turkey and also to assess ceruloplasmin, albumin, and estradiol affect on the change of these parameters during pregnancy.

METHODS

The reference population included 240 serum samples from healthy subjects (120 women and 120 men) ranging between 18 to 80 years. Blood sampling was carried out after 12 hours of fasting. Samples were taken into biochemical tubes without separator gel and were centrifuged at 3000 rpm x 10 minutes. Analyzes were performed on serum using spectrophotometric methods. Statistical analyzes were performed in R (v.4.1.0). All data were initially assessed for normality by applying Shapiro–Wilk test. The Grubb's test was conducted to identify and eliminate outliers in the measured data series. Data were expressed as mean with ± standard deviation (SD), and p value less than 0.05 was considered statistically significant. The parametric method has been used to calculate lower and upper reference limits as 2.5 and 97.5 percentiles of the reference interval distribution. Partial least squares (PLS) path modeling with the mediator and moderator analysis was performed using SmartPLS 3 software.

RESULTS

Serum copper reference intervals were found to be 73.60 - 148.87µg/dL and 56.91 - 143.76 µg/dL in women and men, respectively. Serum zinc reference intervals were measured as 66.40 - 104.67 µg/dL and 65.58 - 111.54 µg/dL in women and men, respectively. A statistically significant correlation was found between estradiol and ceruloplasmin (r=0.498). Serum copper was also significantly correlated with ceruloplasmin ($p < 0.05$). The ceruloplasmin increase in pregnant women was found related to the estradiol effect. However, the most important finding of the current study was revealed by structural equation modeling analysis in the healthy pregnant population. According to this, serum zinc levels decreased due to the loss of albumin in pregnant women while serum copper levels increased due to the induction of ceruloplasmin that was mediated by albumin.

CONCLUSIONS

Being first to use the modeling analysis determining the mediator effect behind the aforementioned interaction, our study brings novelty to the field. Ultimately, this work establishes recent reference intervals of zinc and copper in Turkish population and suggests that albumin levels should be evaluated when investigating the reference intervals of these elements particularly during pregnancy.

Reference intervals and decision limits

W216

BENCHMARKING OF INDIRECT METHODS FOR REFERENCE INTERVAL ESTIMATION

T. Ammer [2], A. Schützenmeister [5], H.U. Prokosch [1], M. Rauh [3], J. Zierk [4], C.M. Rank [5]

[1]*Chair of Medical Informatics, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany*

[2]*Chair of Medical Informatics, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany; Roche Diagnostics GmbH, Penzberg, Germany*

[3]*Department of Pediatrics and Adolescent Medicine, University Hospital Erlangen, Erlangen, Germany*

[4]*Department of Pediatrics and Adolescent Medicine, University Hospital Erlangen, Erlangen, Germany; Center of Medical Information and Communication Technology, University Hospital Erlangen, Erlangen, Germany*

[5]*Roche Diagnostics GmbH, Penzberg, Germany*

BACKGROUND-AIM

Precise reference intervals are required to properly interpret laboratory test results. The current gold standard is to determine the central 95% range of test results from apparently healthy subjects ('direct' method). However, this method is practically, logistically and ethically challenging. Thus, several publications have proposed indirect methods that use routine measurements obtained from laboratory information systems. In order to assess and compare the performance of different algorithms, a standardized and systematic evaluation is needed, which is currently not available.

METHODS

We provide a benchmarking suite for the quantitative comparison of different methods based on 600 simulated test datasets that cover three main distribution types observed in laboratory practice and mimic real-world data: Calcium (normal distribution), Thyroid-stimulating hormone (skewed distribution), and Gamma-Glutamyltransferase (highly skewed distribution). In our simulations, we added pathological distributions with varying location and fraction to the physiological distribution to identify the limitations of the methods. Additionally, we used two different data set sizes (N=5,000 and N=50,000) to quantify the effect of data set size on results. We applied three indirect methods to the simulated data sets, specifically the RLE (Reference Limit Estimator), kosmic and the recently published refineR algorithm. To evaluate their performance, we computed the percentage errors and compared the indirect methods to each other as well as to the simulated direct approach (N=120 and N=400 samples).

RESULTS

For all methods, the distribution type had a strong influence on the results: more skewed distributions resulted in larger percentage errors. Overall, refineR achieved the lowest median of the percentage errors of all test cases (3.04 %), compared to the RLE (11.03%) and kosmic (5.37%), and outperformed the direct method with N=120 (5.62%). Regarding runtime, refineR showed the lowest median runtime (2.46 s), followed by kosmic (4.12 s) and the RLE (15.4 s).

CONCLUSIONS

We present a first step towards a standardized benchmarking suite that enables a quantitative comparison in-between indirect and between indirect and direct methods, ultimately revealing strengths and weaknesses of the different approaches.

Reference intervals and decision limits

W217

CHANGE OF EOSINOPHIL REFERENCE INTERVAL AND ITS IMPACT ON INTERPRETATION OF PATIENT RESULTS

P. Pozaić [1], G. Fressl Juroš [1], M. Jurina [1]
[1]*Department of Clinical-Laboratory Diagnostics, Children's Hospital Srebrnjak, Zagreb, Croatia*

BACKGROUND-AIM

Reference intervals (RIs) are a very important tool for interpretation of laboratory test results and are often adopted from literature, manufacturer's range, official recommendations or guidelines. After discussing with allergy specialist in our institution we decided to change RIs for eosinophil blood count. Therefore, the aim of our study was to assess the change of positive patient number (eosinophil blood count greater than the upper limit of RI (URI)) after changing the reference interval.

METHODS

This study included 95 children. Inclusion criteria were positive absolute and relative eosinophil count (higher than the new URI), presence of the allergic disease and 1 or more positive allergen on skin prick test or allergen-specific immunoglobulin E class ≥1. New RIs currently used are 0-0.30x109/L (<6y); 0-0.20x109/L (≥6y) and 0-3% (<8y; >16y); 0-2 % (8-16y). They were adopted from the Textbook of Hematology, Mckenzie, 2nd edition. Old RIs which are 0-0.70x109/ L (<7y); 0-1.04x109/L (8-19y) and 0-6% (<7y); 0-9% (8-19y) were adopted from recommendations proposed by the Croatian Chamber of Medical Biochemists. Old RIs were applied to all patients to reassess them as positive or negative. McNemar's test was used to test difference between number of positive patients before and after applying RIs with statistical significance $P < 0.05$.

RESULTS

Median for absolute eosinophil count was 0.49(0.42-0.58) x109/L and for relative 6.6 (5.4-7.9)%. With old RIs there would be only 19/95 positive patients or 80% (95% CI 72-88%) difference and 37/95 positive patients or 61% (95% CI 51-71%) difference in absolute and relative eosinophile blood count, respectively. All differences were statistical significant with $P < 0.001$.

CONCLUSIONS

As expected, there was a quite increase in the patient number that has eosinophil count higher than the URI after we started using new RIs. This could be explained by the fact that these are patients that have an allergic disease in the background and such finding is anticipated as well as consistent with the diagnosis. The good communication between laboratory and clinicians is important for the improvement of total testing process especially its post-analytical phase which is a key part of the patient diagnosis.

Reference intervals and decision limits

W218

TESTOSTERONE REFERENCE INTERVALS FOR CASTRATED PROSTATE CANCER PATIENTS ESTABLISHED FOR ONE BEST-PRACTICE METHOD AND FOUR COMMONLY USED IMMUNOASSAYS

L. Van Winden [3], E. Lentjes [4], A. Demir [1], H. Huijgen [2], A. Bergman [5], H. Van Der Poel [6], H. Van Rossum [3]
[1]*Department of Laboratory Medicine, Meander Medical Center, Amersfoort, The Netherlands*
[2]*Department of Laboratory Medicine, Rode Kruis Hospital, Beverwijk, The Netherlands*
[3]*Department of Laboratory Medicine, The Netherlands Cancer Institute, Amsterdam, The Netherlands*
[4]*Department of Laboratory Medicine, University Medical Center Utrecht, Utrecht, The Netherlands*
[5]*Department of Medical Oncology, The Netherlands Cancer Institute, Amsterdam, The Netherlands*
[6]*Department of Urology, The Netherlands Cancer Institute, Amsterdam, The Netherlands*

BACKGROUND-AIM

Chemical castration is an essential treatment for advanced prostate cancer and testosterone testing is relevant for evaluating castration adequacy and diagnosis of castration resistant prostate cancer. Currently, a general testosterone cut-off at 1.7 nmol/L has been established in clinical guidelines to define adequate castration. This cut-off, however, is based on historical and outdated testosterone assays, not necessarily comparable to the present-day automated immunoassays (AIA) generally applied in today's medical laboratories. Therefore, in this study, we determined testosterone reference intervals in castrated prostate cancer patients for 5 testosterone assays: one best-practice liquid-chromatography tandem-mass spectrometry (LC-MS/MS) based assay and four commonly used AIA

METHODS

In this study, we determined testosterone reference intervals in castrated prostate cancer patients for 5 testosterone assays: one best-practice liquid-chromatography tandem-mass spectrometry (LC-MS/MS) based assay and four commonly used AIA. Leftover serum from 120 prostate cancer patients treated with luteinizing hormone-releasing hormone agonists were collected, aliquoted and analysed by all testosterone assays.

RESULTS

Significant differences in testosterone concentrations and the upper limit of the castrated reference interval were observed between the different testosterone methods. The latter (95th percentile) ranged from 0.472 nmol/L for the LC-MS/MS based assay to 1.25 nmol/L for one of the AIA.

CONCLUSIONS

These results suggest that assay-specific testosterone cut-offs may be more appropriate in evaluating the adequacy of castration in prostate cancer patients and diagnosis of castration resistant prostate cancer than a fixed cut-off.

Reference intervals and decision limits

W219

VERIFICATION OF ACTH REFERENCE INTERVAL IN ORAN POPULATION

S. Chellouai [1], a. Besbes [1], a. Mial [2], h. Louali [2], f. Si Larbi [2], a. Saadi-Ouslim [1]
[1]*Biochemistry Departement, University Hospital of Oran «1er Novembre 1954», BP N°4166 Ibn Rochd, Oran, Algeria.*
[2]*Departement of Pharmacy, Faculty of Medecine, University of Oran, BP 1510 El M'Naouer31100 Oran, Algeria*

BACKGROUND-AIM

The reference interval is a fundamental tool for interpreting results of biological tests, it is part of the standardization and harmonization of results reporting recommended by the ISO norm 15189.

METHODS

We carried out a study which main objective is to validate the transferability of the reference interval of ACTH provided by the reagent manufacturer on the population served at the biochemistry laboratory of the EHU November 1st of Oran, according to the recommendations of IFCC-LM and CLSI C28-A3.
The ACTH assay was performed on the IMMULITE 2000 XPI® system in 20 apparently healthy adult subjects.

RESULTS

Reference values obtained on 20 samples were within the reference interval of the supplier (<46pg / ml), no value exceeded this limit, which allowed us to validate the transferability of the interval of the supplier's reference on the population served by the EHU biochemistry laboratory November 1, Oran.

CONCLUSIONS

In conclusion, the interpretation of the results of medical biology examinations is complex, we are also convinced that the work carried out is only a primary step for future studies including the establishment of the reference interval of our own laboratory

Reference intervals and decision limits

W220

ASSESSMENT OF C-REACTIVE PROTEIN AND INTERLEUKIN-6 LEVELS TO ESTABLISH DECISION LIMITS IN PATIENTS INFECTED BY SARS-COV-2

M. Mayor-Reyes [1], R. Mondéjar-García [1], C. Cañavate-Solano [1], L. Diez-Herranz [1], J.D. Santotoribio [1]
[1]*Department of Clinical Laboratory, Puerto Real University Hospital, Cadiz*

BACKGROUND-AIM

Owing to the pandemic caused by SARS-CoV-2, Medical Laboratories have implemented new biomarkers to facilitate the diagnosis and consequently the election of treatment. Interleukin 6 (IL-6) is an inflammatory cytokine involved in the cytokine storm induced by SARS-CoV-2. In addition, IL-6 is a biomarker which is related to severity of disease and severe acute respiratory distress syndrome. A biological immunosuppressive drug as Tocilizumab is a recombinant monoclonal antibody directed against IL-6 receptor, which has been shown to reverse cytokine storm. Generally, Tocilizumab is recommended when IL-6 is upper o equal than 40 pg/mL in adults. The aim of this study was to assess the association between C-reactive protein (CRP) and IL-6 with the purpose of establishing biochemical criteria for performing IL6.

METHODS

This is a retrospective and observational study with the following criteria of selection: Hospitalized patients with SARS-CoV-2 infection between the period from March 2020 to December 2020 and biomarkers (CPR, IL-6) requested in the serum sample on the day of admission.
IL-6 was analized by electrochemiluminescence immunoassay (ECLIA) in Cobas e411(Roche®) autoautoanalizer. CPR was determined by immunoturdidimetric assay in the Alinity c analizer (Abbott®). Reference values in serum for IL-6 y CRP were lower than 7 pg/mL and lower than 5mg/L, respectively. It was collected the following variables: gender, age and biomarkers (IL-6 y CRP), with the support of Laboratory Informatic System. Data was processed by statistical program Medcalc®, where p<0,05 was considered significant. A statistical correlation study (rho) and a Receiver Operating Characteristic curve (ROC) were performed to determine the diagnostic utility of CRP in the IL-6 application.

RESULTS

The variables studied followed a non-Gaussian distribution (D'Agostino-Pearson test reject normality (P<0,0001)). A total of 103 patients, 42 women and 61 men, were included with a median age of 67 years old [32-93] The serum levels of IL-6 with a median of 32.6 pg/mL [1.97-5000.00]; and CRP with a median of 77.70 mg / L [5.98-337.00]. No statistically significant differences were found between men and women with CRP and IL-6 determined in serum, neither was there a significant correlation between the age of the patients and the biochemical values..
The Spearman's coefficient of rank correlation (rho) between IL-6 and CRP was 0.460 (p <0.0001) 95% CI from 0,29 to 0,60. The area under the ROC curve of CRP to predict patients with IL-6 > 40.00 was 0.714 (p <0.0001) 95% CI from 0.616 to 0.799. Our results showed that 95% of the patients with serum IL-6 levels ≥ 40.00pg / mL had serum CRP levels > 20.0 mg / L.

CONCLUSIONS

Therefore, serum CRP showed almost moderately strong positive correlation with serum IL-6 levels. So could be used as a criterion for the determination of IL-6 in patients infected by SARS-CoV-2 as candidates for treatment with tocilizumab. It should be noted that all the patients included in our study had a CRP above the reference value. The cut off obtained agree with that recently published in the bibliography. It is necessary to increase the number of study patients to improve our results.

Reference intervals and decision limits

W221

URINARY FREE CORTISOL – ESTIMATING THE UPPER REFERENCE LIMIT IN CROATIAN POPULATION ON THE ABBOTT ARCHITECT I2000

I. Zec [2], A. Bokulić [2], I. Kruljac [1]
[1]*Department of Endocrinology, Poliklinika Solmed, Zagreb, Croatia*
[2]*Laboratory of Endocrinology, Sestre Milosrdnice University Hospital Center, Zagreb, Croatia*

BACKGROUND-AIM

Urinary free cortisol in the 24-hour urine sample (UFC) is one of the three initial tests for evaluating hypercortisolism. Setting up the accurate upper reference limit (URL) for UFC is essential for the diagnosis of Cushing syndrome (CS). Liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS) is considered the most accurate method to determine the UFC. Despite the limitations, immunoassays are still common methods for UFC measurement. Abbott Architect i2000 immunoassay is comparable to the LC-MS/MS method with a slight overestimation of UFC concentration. According to the manufacturer, the 95% reference interval (RI) is derived from apparently healthy individuals, ranging from 12 to 486 nmol/24h. This study aimed to calculate 95% URL for the Croatian population.

METHODS

Samples are collected from 234 apparently healthy individuals, 116 women and 118 men. Participants with a known disease of the hypothalamic-pituitary-adrenal axis, renal diseases, glucocorticoid administration and acute illness are excluded from the analysis. After collection, 24-hour urine samples are centrifuged and cortisol is analysed on Abbott Architect i2000. The URL is estimated with the nonparametric method as right-sided, 95% RI with the calculation of 90% confidence interval (90%CI). The extreme values are tested with the Tukey method. Summary statistics are presented as median and IQR.

RESULTS

Median age of all subjects is 50 (33 – 65) years. Median volume of 24-hour urine is 1825 (1300 – 2300) mL. The calculated URL is 245 nmol/24h (90%CI: 214-269 nmol/24h). UFC concentrations for nine participants are recognized as outliers by Tukey method, but still included in the calculation. Median of UFC in men and women is 110 nmol/24h and 90 nmol/24h, respectively.

CONCLUSIONS

The estimated URL in the Croatian population is significantly lower than the upper limit of reference interval declared by the manufacturer (245 nmol/24h vs 486 nmol/24h). However, the URL refers to healthy subjects, thus cannot be mistaken for the cut off value for the diagnosis of CS.

Reference intervals and decision limits

W222

INDIRECT REFERENCE INTERVAL OF SERUM FREE THYROXINE CONCENTRATIONS ESTIMATED USING LABORATORY "BIG DATA".

E. Tejedor Hernández [1], I. Cachón Suárez [1], R. Navarro Badal [1], M.J. Castro Castro [1], J. Ros Pau [1], R. Ruiz Morer [1], A. Arbiol Roca [1], D. Dot Bach [1]
[1]*Laboratori Clínic Territorial Metropolitana Sud, Hospital Universitari de Bellvitge, Barcelona.*

BACKGROUND-AIM

Thyrotropin (TSH) and free thyroxine (FT4) serum concentrations are the most used laboratory tests to diagnose thyroid dysfunctions. Use of non appropriate reference intervals (RIs) could lead to errors in identifying thyroid dysfunction where no pathology exists and potentially cause thyroid abnormalities to be overlooked.
The aim is to establish the RI of FT4 by an Indirect-method (IM), using data collected at the laboratory information system (LIS) and verifying their applicability.

METHODS

Data were selected between November 2019 to May 2021 at the Laboratori Clinic Territorial Metropolitana Sud, Barcelona. A total of 119263 results of FT4 serum concentrations were retrieved; this sample group was reduced to 15296 results, after selection criteria applied. Only results from outpatients in a primary care setting and with a single result on database were included. Results from: younger than 18 years, pregnant women, patients coming from endocrinology department, individuals with TSH results outside the RI or with positive antithyroid antibodies (AA), were excluded.
All FT4 tests were measured using Cobas 8000-e801 analyzers (Roche Diagnostics) and the analytical method has been stable during the period of data collection. Statistical analysis was performed on Analyse-it® (Microsoft Excel). After outliers were excluded (Tukey method), the reference limits (RL) of FT4 serum concentrations were calculated using a non-parametric approach, as the 2,5 and 97,5 percentiles, with a 90% confidential intervals.
To validate the new RI, according to CLSI EP28-A3 guideline, 27 reference individuals were selected. In all of them, serum TSH, FT4 and AA were measured. Individuals with positive AA were excluded.

RESULTS

The lower and upper RL for FT4 serum concentrations were 10.9 (10.8-11.0) pmol/L and 20.3 (20.2-20.4) pmol/L, respectively. The median of the polulation was 15.1 mol/L.
In the reference individuals group, 6 individuals were excluded and one outlier result was identified (Dixon test). The 20 remaining results were inside the RI estimated.

CONCLUSIONS

It is verified that the new RI created with big data fits our population under the recommendations published in this topic. IMs are a promising tool for laboratories to develop cheap, specific and updated RIs.

Reference intervals and decision limits

W223

REFERENCE VALUES FOR SCALP HAIR CORTISOL, CORTISONE, DHEA, AND TESTOSTERONE BY ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY IN HEALTHY YOUNG MAN COHORT

A. Mažeikienė [2], D. Karčiauskaitė [2], E. Mazgelytė [2], T. Petrėnas [1], A. Kaminskas [1], J. Songailienė [1], A. Utkus [1]

[1]*Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania*

[2]*Department of Physiology, Biochemistry, Microbiology and Laboratory Medicine, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania*

BACKGROUND-AIM

Human scalp hair is a reasonable matrix for determining long-term steroid hormone concentrations. However, the reference values for hair steroid hormones measured by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) are lacking. This study aimed to evaluate hair cortisol, cortisone, testosterone, and dehydroepiandrosterone (DHEA) levels using UHPLC-MS/MS method in a healthy young man cohort.

METHODS

A total of 100 healthy young men aged 18-26 were enrolled in the longitudinal study. Hair sampling was repeated three times in 6 months period with the following assessment points: T1, T2 (+3 months), T3 (+3 months). Steroid hormone concentrations were determined from the first centimeter of hair proximal to the scalp by a UHPLC system (Shimadzu Corporation, Kyoto, Japan) coupled with a triple quadrupole tandem mass spectrometer (LCMS-8060, Shimadzu). Data acquisition was performed by Shimadzu LabSolutions software (version 1.20). IBM SPSS 27 version was used for statistical analysis.

RESULTS

The minimum, 25th, 50th, 75th percentile, and maximum hair steroid hormone concentrations were: cortisol 1.54, 2.51, 3.29, 4.61, and 12.53; cortisone 7.04, 10.74, 12.87, 16.43, and 32.77; testosterone 0.21, 0.38, 0.50, 0.69, and 1.96; DHEA 2.98, 7.67, 11.42, 15.43, and 76.83 ng/g respectively.

CONCLUSIONS

The establishment of hair cortisol, cortisone, DHEA, and testosterone reference ranges broadens the potential applications of these biomarkers in research and clinical care.

Funding: This research was funded by the Research Council of Lithuania (LMTLT) under project agreement No S-MIP-20-59

Reference intervals and decision limits

W224

PEDIATRIC REFERENCE INTERVALS FOR SERUM CALPROTECTIN IN THE CALIPER COHORT OF HEALTHY CHILDREN AND ADOLESCENTS: A POTENTIAL BIOMARKER FOR NEONATAL AND PEDIATRIC SEPSIS

M.K. Bohn [1], S. Wilson [1], L. Sepiashvili [1], K. Adeli [1]
[1]*The Hospital for Sick Children, Toronto, Canada*

BACKGROUND-AIM

Calprotectin is an antimicrobial peptide that is released from granulocytes and mononuclear phagocytes immediately after host-pathogen interaction. Measurement of serum calprotectin in neonates and children suspected with sepsis has been suggested to improve outcomes due to higher diagnostic sensitivity relative to other markers of infection (e.g. C-reactive protein, procalcitonin). In the current study, healthy neonates and children were recruited as part of the Canadian Laboratory Initiative Paediatric Reference Intervals (CALIPER) to establish robust reference intervals for serum calprotectin.

METHODS

The Gentian Calprotectin Immunoassay was validated on the Abbott ARCHITECT platform at The Hospital for Sick Children, including precision and linearity as per Clinical and Laboratory Standards Institute (CLSI) guidelines. 263 healthy children and adolescents were recruited with informed consent through community initiatives. Health status was assessed through health questionnaire and serum was collected and tested for calprotectin using the Gentian assay. Age and sex-specific differences were assessed using the Harris & Boyd method and reference intervals were then calculated as per CLSI guidelines.

RESULTS

The Gentian Calprotectin Immunoassay demonstrated a total precision of 7.5% for level 1 (mean: 1.04 mg/L) and 1.5% for level 2 (mean: 10.1 mg/L). It was determined to be linear across the analytical reporting range (slope: 0.97, intercept: 0.12, R2: 0.999, concentration range: 0-19 mg/L). No age- or sex-specific differences were observed, demonstrating a consistent distribution from birth to adolescence. Many pediatrics values were below the limit of detection of the assay (0.15 mg/L). One reference interval of <2.97 mg/L (90% confidence interval: 2.66, 3.23) was established.

CONCLUSIONS

In the current study, pediatric reference intervals for serum calprotectin were established for the first time in the CALIPER cohort. Pediatric serum calprotectin levels did not demonstrate significant age- or sex-specific differences. These data will be useful to pediatric institutions considering implementing serum calprotectin in inflammatory disease monitoring as well as providing baseline information for clinical studies investigating septic patients.

Reference intervals and decision limits

W225

PEDIATRIC REFERENCE INTERVAL ESTABLISHMENT FOR 32 HEMATOLOGY PARAMETERS ON THE MINDRAY BC6800 PLUS SYSTEM

M.K. Bohn [1], S. Wilson [1], G. Fabiano [1], K. Adeli [1]
[1]*The Hospital for Sick Children, Toronto, Canada*

BACKGROUND-AIM

Hematological parameters vary significantly throughout growth and development due to physiological processes such as fetal-to-adult erythropoiesis and puberty. Pediatric age- and sex-specific reference intervals are thus needed for appropriate clinical decision-making. In the current study, blood samples from children and adolescents were collected as part of the Canadian Laboratory Initiative Paediatric Reference Intervals (CALIPER) to establish pediatric reference intervals on the Mindray BC6800 Plus for the first time.

METHODS

The Mindray BC6800 Plus was validated at The Hospital for Sick Children, including precision and method comparison to Sysmex XN-3000 system for main complete blood count parameters. Approximately 200 children and adolescents from birth to 18 years of age were enrolled in the study. Whole blood was collected through KEDTA vacutainers and run on the Mindray BC6800 Plus for 32 in vitro diagnostics parameters. Preliminary reference intervals were established as per Clinical and Laboratory Standards Institute Guidelines.

RESULTS

All hematological parameters on the Mindray BC6800 Plus demonstrated acceptable precision and most demonstrated excellent concordance to the Sysmex XN-3000 with Pearson's R>0.9 (exceptions included: mean corpuscular hemoglobin concentration and monocyte count). In pediatric study cohort, three reference value distribution patterns were observed: 1) sex-specific differences in adolescence with males demonstrating higher levels relative to females (e.g. RBC count, hemoglobin); 2) increasing or decreasing trends throughout the pediatric age (e.g. WBC and platelet count); and 3) consistent reference value distributions from birth to adolescence (e.g. mean corpuscular hemoglobin and basophil count).

CONCLUSIONS

In the current study, pediatric reference intervals for several hematology parameters on the Mindray BC6800 Plus were established. Pediatric reference value distributions confirmed previously published dynamics, necessitating age- and sex-specific consideration in interpretation. Further sample analysis is needed to improve robustness of reference interval estimates, particularly in the neonatal period.

Reference intervals and decision limits

W226

PEDIATRIC REFERENCE INTERVALS FOR TRACE ELEMENTS IN THE CALIPER COHORT OF HEALTHY CHILDREN AND ADOLESCENTS USING ICP-MS/MS AND HR-MS TECHNOLOGY

M.K. Bohn [3], M. Nichols [1], L. Wang [1], V. Bhayana [1], J. Macri [2], K. Adeli [3]
[1]*London Health Sciences Centre, London, Canada*
[2]*McMaster University, Hamilton, Canada*
[3]*The Hospital for Sick Children, Toronto, Canada*

BACKGROUND-AIM

Essential trace elements play critical roles in cellular metabolism and neurocognitive function. Elemental deficiencies have serious implications, particularly in pediatrics wherein the risk of developing such deficiencies is higher. Biomonitoring of heavy metals in children is also critical to assess toxicity. Pediatric reference intervals (RIs) for trace elements and heavy metals are lacking on modern analytical systems. In the current study, reference intervals were established for 14 plasma and 22 whole blood trace elements in the CALIPER cohort of healthy children and adolescents.

METHODS

Approximately 300 healthy children and adolescents were recruited with informed consent. Participation required completion of health questionnaire, and blood donation (Royal Blue Top tubes, BD Vacutainer). Trace elements and heavy metals were measured in whole blood and plasma samples using two technologies: 1) ICP-MS/MS (Hamilton Health Sciences, n=175), 2) HR-MS (London Health Sciences, n=160) where possible. Pediatric reference value concentrations were evaluated for age and sex-specific differences using the Harris and Boyd method. Reference intervals were then established according to Clinical and Laboratory Standards Institute Guidelines.

RESULTS

Of all elements and heavy metals assessed, none required sex partitioning and 10 required age partitioning (e.g. copper, manganese, cadmium). Reference value distributions determined via ICP-MS/MS and HR-MS demonstrated excellent concordance, with few exceptions (e.g. molybdenum, cobalt, nickel). Few analytes assessed demonstrated extremely low values near or below the limit of detection, including platinum and silver.

CONCLUSIONS

This study is the first to report pediatric reference intervals for a comprehensive trace element panel using both ICP-MS/MS and HR-MS technology. Study findings suggest some trace elements require age-specific interpretation for appropriate clinical decision-making. Evaluated methods were also relatively concordant for most assays, supporting the feasibility of common reference intervals in pediatrics.

Reference intervals and decision limits

W227

DEFINING TRIMESTER-SPECIFIC REFERENCE INTERVALS FOR CARBOHYDRATE DEFICIENT TRANSFERRIN IN PREGNANT WOMEN.

E. Göttgens [3], L. Haverkate [1], M. Langelaan [3], S. Lunshof [2], T. Ermens [3], L. Jacobs [1]
[1]*Department of Clinical Chemistry and Hematology, MeanderMC, Amersfoort, The Netherlands*
[2]*Department of Gynaecology, Amphia Hospital, Breda, The Netherlands*
[3]*Result Laboratory for Clinical Chemistry and Hematology, Amphia Hospital, Breda, The Netherlands*

BACKGROUND-AIM

Extensive consumption of alcohol during pregnancy can lead to severe complications for the unborn child. Therefore, an objective unbiased screening method is necessary to detect excessive intake of alcohol during pregnancy. Carbohydrate-deficient transferrin (CDT) levels in serum have become a common biomarker for long-term alcohol consumption. However, several studies showed that CDT levels are elevated during pregnancy, for reasons unrelated to alcohol intake. The aim of this study is to investigate the changes in CDT values during pregnancy and to determine trimester-dependent reference values.

METHODS

439 serum samples of 147 healthy pregnant women were obtained during different trimesters as well as post-partum and were analysed by high-performance liquid chromatography (HPLC) and N-Latex immunonephelometric assay. Reference intervals were established per trimester.

RESULTS

This study demonstrates a trimester-dependent increase of CDT levels for both the HPLC-based method and the N-Latex immunoassay. The estimated upper reference values for CDT analysis were 1.55%, 1.96%, 2.05% and 1.35% for trimester 1, 2 , 3 and four weeks post-partum for the HPLC-method and 1.50%, 1.74%, 1.74% and 1.42% for the N-Latex immunoassay. Related to commonly used reference-intervals and cut-off values (1.7% CDT), this would lead to 24.8% and 39.4% false positive cases in trimesters 2 and 3 respectively.

CONCLUSIONS

We demonstrate that CDT levels rise during pregnancy and have established trimester-specific reference intervals to prevent false-positive results in alcohol abuse screening tests.

Reference intervals and decision limits

W228

EVALUATION OF REFERENCE INTERVALS FOR CLASSICAL (CH50) AND ALTERNATIVE PATHWAY (AP50) FUNCTIONAL COMPLEMENT ASSAYS.

S. Capiau [1], J. Delanghe [1], P. De Kesel [1]
[1]*Department of Laboratory Medicine, Ghent University Hospital, Ghent, Belgium*

BACKGROUND-AIM

Although vendors have to provide appropriate reference intervals (RIs), critical evaluation remains important. Here, the validity of the Wieslab Complement system Alternative pathway kit (EuroDiagnostica, Sweden) and the Wako Autokit CH50 (Wako, Germany) RIs was assessed.

METHODS

Serum samples were obtained from 100 healthy volunteers and stored at -80°C until analysis. AP50 results were read out on a Tecan Sunrise (Tecan, Switzerland) and CH50 was measured on Cobas c502 (Roche diagnostics, Germany). Whenever CH50 exceeded the measurement range of 60 U/mL (n=70) samples were diluted 1:2 with saline or heat inactivated pooled serum and reanalyzed, as recommended in literature.

RESULTS

The used dilution strategies yielded inaccurate results. As CH50 values >60 U/mL are outside of the measurement range, results after dilution are inaccurate, and a RI cannot be solely based on a non-representative part of the reference population (i.e. only samples ≤60 U/mL), the CH50 RI was calculated using values obtained after 1:2 dilution with saline, divided by 0.68 (experimentally determined correction factor). For AP50 a double-sided 95% RI was calculated. For CH50 a left-sided 97.5% RI was determined, given the abovementioned issues and the fact that mainly decreased CH50 activities are relevant. The obtained RIs with corresponding 90% confidence intervals were 31 [26-37] to 106 [100-112] % for AP50 and 36.9 [34.9-39.2] U/mL for CH50. The AP50 RI was similar to the manufacturer's (i.e. 30-113%), whilst the CH50 lower reference limit (LRL) was substantially higher than the manufacturer's (i.e. 31.6 U/mL). Spiking experiments revealed CH50 is decreased starting from a C3 (C4) concentration corresponding to about 22% (31%) of the C3 (C4) LRL when using the manufacturer's CH50 LRL, or to about 31% (40%) of the C3 (C4) LRL when using the in house CH50 LRL. In 490 retrospectively collected, consecutive CH50 results, 9 additional patients would have been classified as having a reduced CH50 using the in house CH50 LRL. A decreased C3 and/or C4 was observed in 7/9 patients and all had medical conditions associated with reduced CH50 activity.

CONCLUSIONS

For the Wieslab AP50 kit the manufacturer's RI proved applicable to our population. For the Wako CH50 kit a higher LRL was found and the validity of this in house established LRL of 36.9 U/mL was demonstrated.

Reference intervals and decision limits

W229

WITHIN-SUBJECT AND BETWEEN-SUBJECT BIOLOGICAL VARIATION ESTIMATES OF GLUTAMINE, TYROSINE, PHENYLALANINE, LEUCINE AND CITRULLINE IN HEALTHY SUBJECTS

A. Coşkun [3], O. Demirelce [1], A. Carobene [9], A.K. Aarsand [6], M. Serteser [3], S. Sandberg [6], J. Díaz-Garzón [5], P. Fernandez-Calle [5], M. Erkaya [2], C. Coskun [8], M.M. Yekrek [7], E.N. Eroğlu [2], H. Dağ [2], N. Jonker [4], F.B. Aksungar [3], I. Unsal [1]

[1]*Acibadem Labmed Clinical Laboratories, Atasehir, Istanbul, Turkey,*

[2]*Acibadem Mehmet Ali Aydınlar University, School of Medicine, Atasehir, Istanbul, Turkey*

[3]*Acibadem Mehmet Ali Aydınlar University, School of Medicine, Department of Medical Biochemistry Atasehir, Istanbul, Turkey,*

[4]*Certe, Wilhelmina Ziekenhuis Assen, Assen, The Netherlands*

[5]*Department of Laboratory Medicine, La Paz University Hospital, Madrid, Spain; and Analytical Quality Commission, Spanish Society of Laboratory Medicine (SEQCML), Barcelona, Spain*

[6]*Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital, Bergen, Norway.*

[7]*Haydarpaşa Numune Research and Training Hospital, Department of Medical Biochemistry Kadiköy, Istanbul, Turkey*

[8]*Istanbul Basaksehir Çam and Sakura City Hospital, Department of Medical Biochemistry Bahçesehir, Istanbul, Turkey*

[9]*Laboratory Medicine, IRCCS San Raffaele Scientific Institute, Milan, Italy*

BACKGROUND-AIM

Amino acids are structural units of proteins and also needed for vital processes such as biosynthesis of hormones and neurotransmitters. Serum amino acids levels are applied in the diagnosis and monitoring of patients with inborn errors and nutritional statues in e.g. chronic liver and renal diseases. Reliable biological variation (BV) data is required for the safe clinical application of amino acids. The aim of this study was to determine within-subject (CV_I) and between-subject (CV_G) BV estimates for glutamine, tyrosine, phenylalanine, leucine and citrulline.

METHODS

Serum samples were collected from 66 healthy volunteers (35 females and 31 males) for 10 consecutive weeks and stored at -80°C prior to analysis. Serum glutamine, tyrosine, phenylalanine, leucine and citrulline concentrations were measured using liquid chromatography–tandem mass spectrometry (LC-MS/MS). The measurement results were assessed for outliers, trends and variance homogeneity following BIVAC criteria. CV_I and CV_G estimates were calculated using CV-ANOVA and ANOVA on log-transformed data, respectively.

RESULTS

The CV_I (95% CI) estimates were 11.7% (11.0–12.5), 14.5% (13.6–15.6), 18.7% (17.6–20.0), 13.5% (12.6–14.4) and 19.1% (17.9–20.4) for glutamine, tyrosine, phenylalanine, leucine and citrulline, respectively. CV_I estimates for male and female subjects were significantly different for all amino acids except phenylalanine. The indices of individuality ranged from 0.85 (leucine) to 1.68 (phenylalanine).

CONCLUSIONS

This study, based on a rigorous protocol, provides more stringent and updated BV data for important amino acids. Due to the differences of CV_I between males and females, sex stratification would be necessary for monitoring patients' consecutive results for glutamine, tyrosine, leucine and citrulline.

Reference intervals and decision limits

W230

THE ROLE OF ALDOSTERONE/RENIN RATIO DETERMINATION IN DIAGNOSIS OF PRIMARY ALDOSTERONISM

R. Mijovic [2], B. Milicic [1], S. Nikolic [2]
[1]*University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia*
[2]*University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia Clinical Center of Vojvodina, Serbia*

BACKGROUND-AIM

The aim of this retrospective study was to evaluate the role of the aldosterone/renin ratio (ARR) in the diagnosis of primary aldosteronism.

METHODS

This retrospective study included a total of 362 hypertensive patients evaluated for presence of primary aldosteronism. According to the data collected from the medical history (concentrations of potassium, sodium, total and ionized calcium, bicarbonate and blood pH, imaging diagnostic procedure as computed tomography and magnetic resonance and data on possible surgery and pathohistological findings) and obtained values of aldosterone, renin and ARR, patients were divided into three groups: patients with primary aldosteronism (n=37), secondary aldosteronism (n=76) and a group of hypertensive patients with a normal value of renin and aldosterone (n=249). Total renin and aldosterone concentrations were determined by ELISA (enzyme linked immunosorbent assay) kit for quantitative in vitro diagnostic measurement in plasma, IBL International.

RESULTS

Concentration of renin was significantly lower in patients with primary aldosteronism compared to hypertensive patients (4.08±0.49vs.7.83±8.19;$p<0.05$) and patients with secondary aldosteronism (4.08±0.49vs.54.87±40.90;$p<0.01$). Concentration of aldosterone was significantly higher in patients with primary aldosteronism than in hypertensive patients (231.28±138.91vs.104.46±39.15;$p<0.01$) but with no significant difference regarding the secondary aldosteronism group (231.28±138.91vs.233.05±179.81;$p>0.05$). ARR was significantly higher in patients with primary aldosteronism compared to hypertensive patients (56.79±34.55vs.20.11±11.30;$p<0.01$) and patients with secondary aldosteronism (56.79±34.55vs.8.13±8.25;$p<0.01$). According to the ROC analysis, the cut-off value of 35 for ARR gives a 94% sensitivity, 91% specificity and accomplishes 91% accuracy in the evaluation of primary aldosteronism.

CONCLUSIONS

In our examined group of hypertensive patients, the aldosterone/renin ratio has been proven to be a good screening test with a high diagnostic value in the detection of primary aldosteronism and for defining the level of functional renin/aldosterone axis compared to the individual determination of renin and aldosterone.

Reference intervals and decision limits

W231

AN ESTIMATION OF WHITE BLOOD CELLS REFERENCE INTERVALS IN 0-3 DAY NEONATES USING THE INDIRECT METHOD

L. Saveliev [5], O. Plekhanova [3], N. Eliseeva [2], O. Kalacheva [1], S. Tsvirenko [4]

[1]*Department of clinical laboratory diagnostics, JSC Medsi Group, Moscow, Russia*

[2]*Department of clinical laboratory diagnostics, Regional Children Hospital, Yekaterinburg, Russia*

[3]*Department of clinical laboratory diagnostics, Ural State Medical University, Yekaterinburg, Russia; Department of clinical laboratory diagnostics, JSC Medsi Group, Moscow, Russia*

[4]*Department of clinical laboratory diagnostics, Ural State Medical University; Yekaterinburg, Russia; Department of clinical laboratory diagnostics, Regional Children Hospital, Yekaterinburg, Russia.*

[5]*Department of clinical laboratory diagnostics, Ural State Medical University; Yekaterinburg, Russia; Research Institute of Medical Cell Technologies, Yekaterinburg; Department of CDL, Regional Children Hospital, Yekaterinburg, Russia*

BACKGROUND-AIM

The use of indirect methods of reference intervals (RI) estimating is important in groups that are hard to recruit for direct methods use.
The aim of the study was to estimate the stability of RI calculated of indirect method TMC (Truncated Minimum chi-square) using data from a laboratory database and simulations with different fractions of pathological values.

METHODS

RIs of WBC count from 809 healthy patients' data (0-3 days' neonates) during 2020 from the database were estimated with TMC method in R environment.
The pathological data of 10-50% of normal distribution of pathological WBC data (high, low and both) with different distance from median (1, 1.5, and 2 SD from median of healthy subjects with 0.5-1.5 SD of pathological distributions) was simulated. The same percentages of pathological data were randomly generated. The obtained RI were compared with "true" RIs of healthy patients' data (2,5th and 97,5th percentiles, Tukey test outliers' exclusion).
Finally, RI of 1115 samples of own laboratory data (306 pathological and 809 non-pathological samples) were calculated.
Total error (+/-1TE), and permissible difference (pD) were used for RI verification.

RESULTS

For randomly generated data, the RIs estimated were stable up to 50% added of the pathological data on both sides of the distribution (TE method). There was no agreement with pD value at all.
+-1SD data showed an agreement with "true" method in up to +50%, with TE method; in pD estimation there was an agreement up to 20% at lower end and 50 % at a high end. The best match of RIs was in addition of pathological values to both ends.
In +-2SD pathological data, an agreement was less seen in lower ends in up to +50 %. The result of the simulations at upper limit and at both limits using TE method was acceptable, unlike in pD estimation (up to 10% at both ends).
Addition of 1.5 SD, showed a better agreement in TE comparison at high levels and both (up to 50%, and up to 20% in lower level RI). pD estimation was not so successful (up to 10% in all cases).
The RI estimation of the data from the own laboratory database failed at the lower RI, because of the large left shift.

CONCLUSIONS

The indirect methods should be studied more with laboratory data use. Besides, different approaches to RI acceptability assess can give controversive results.

Reference intervals and decision limits

W232

NUMBER-2: THE AUTOMATION AND EXTENSION TO ROUTINE HAEMATOLOGY OF THE DUTCH INDIRECT DATA-MINING APPROACH TO ESTABLISH POPULATION-SPECIFIC REFERENCE INTERVALS

N. Brouwer [4], C.M. Cobbaert [3], L.M. Martinez [5], M. Van Schrojenstein-Lantman [6], M.H. Thelen [6], F. Rodríguez-Frias [2], W.P. Den Elzen [1]

[1]*Atalmedial Diagnostics Centre, Amsterdam, The Netherlands. // Department of Clinical Chemistry, Amsterdam University Medical Centre, Amsterdam, The Netherlands*

[2]*Biochemistry Department, Clinical Laboratories, Vall d'Hebron University Hospital, Barcelona, Spain. // Department of Biochemistry and Molecular Biology, Autonomic University of Barcelona, Bellaterra, Spain*

[3]*Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Centre. Leiden, The Netherlands*

[4]*Diagnost-IQ, Laboratory for Clinical Chemistry and Hematology, Hoorn, The Netherlands*

[5]*Leiden University Medical Centre, The Netherlands. // Vall d'Hebron University Hospital // Autonomic University of Barcelona, Spain*

[6]*Radboud University Medical Centre. // Result Lab for Clin. Chemistry and Hematology, Amphia Hospital. // Dutch Foundation for Quality Assessment in Medical Laboratories, The Netherlands*

BACKGROUND-AIM

After the first NUMBER (Nederlandse UniforMe BEslisgrenzen en Referentieintervallen) initiative, focussing on chemistry tests, we initiated NUMBER-2, to set up a sustainable system for determination and long-term monitoring of harmonized haematology reference intervals (RI) in the Netherlands using the indirect approach. Parallel to this, we have developed a method to automate the periodic evaluation of the RIs based on the data from only those laboratories that meet performance criteria in the external quality assessment (EQA) in the data collection period.

METHODS

We included medical tests from the Dutch EQA programme 'SKML Hemocytometry'. We extracted anonymous test results from laboratory databases of primary care patients from 10 laboratories using four analytical platforms (Sysmex, Beckman-Coulter, Abbott and Siemens). Results were included when EQA analytical performance specifications were met and medically relatable parameters (e.g. ferritin) were within reference range or not requested. Results were excluded when phlebotomy was performed at home. Per laboratory, per test, outliers were excluded (Tukey method), data were transformed to a normal distribution (if necessary) and means and standard deviations (SDs) were calculated. Then, average means and SDs were calculated to generate pooled (mean±2SD) reference intervals, stratified by age and sex, if necessary.

RESULTS

The analysed data from the red blood cells (erythrocytes, hemoglobin, MCV and MCHC) were very consistent with the current RI used in the Netherlands. Similar results were found for each platform. However, for the different white blood cells types (leukocytes, lymphocytes, neutrophils, monocytes and eosinophils) we found some inconsistencies compared to the current RI or between labs. Additional exclusion criteria were necessary to define the RI for the white blood cell differentiation. In our expert workshop the results were discussed.

CONCLUSIONS

Harmonized RI for the haematology parameters were established. With the network and database at our national EQA organisation, SKML, we have developed an unique platform to establish sustainable nationwide standardized or harmonized RIs. Our holistic approach enables data exchange for basic tests among Dutch healthcare institutions, supports remote monitoring of patients and avoids repeated lab testing.

Reference intervals and decision limits

W233

REFERENCE INTERVALS FOR SYSMEX XN HEMATOLOGICAL PARAMETERS AS ASSESSED IN THE DUTCH LIFELINES COHORT

J. Van Pelt [2], S. Klatte [3], T. Hwandih [3], A. Barcaru [2], I. Riphagen [2], J. Linssen [3], S. Bakker [1]

[1]*Department of Internal Medicine, Division of Nephrology, University of Groningen, University Medical Center Groningen, the Netherlands*

[2]*Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, the Netherlands*

[3]*Medical Scientific Department, Sysmex Europe GmbH, Norderstedt, Germany*

BACKGROUND-AIM

Our aim was to derive reference intervals for all Sysmex XN hematology analyzer parameters. The rationale behind the study was the lack of reference intervals for the XN analyzer specific parameters.

METHODS

Fresh fasting blood samples from 18484 participants in the Dutch Lifelines study were analyzed using two automated XN analyzers. Structured health questionnaires were used to select a subgroup of 15803 apparently healthy individuals for inclusion in the reference population. The technique of Latent Abnormal Values Exclusion (LAVE) was used to reduce the influence of latent disease in the reference population on the resulting reference intervals. We applied analysis of variance to judge the need for partitioning of the reference intervals by sex or age.

RESULTS

We report reference intervals for 65 XN analyzer hematological parameters with and without applying the LAVE concept. Sex-related partitioning was required for red blood cells and hemoglobin (RBC, RBC-O, HGB, HGB-O), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), and the reticulocyte production index (RPI). Partitioning for age was not warranted. BMI and smoking had moderate influence on a minority of the parameters.

CONCLUSIONS

We provide reference intervals for all Sysmex XN analyzer parameters, using a direct approach in a large cohort in the Netherlands.

Reference intervals and decision limits

W234

ESTIMATION OF REFERENCE INTERVALS FOR TSH, FT3, AND FT4 ON ALINITY I FOR TWO REGIONALLY SEPARATED LABORATORIES IN GERMANY: COMPARISON OF THREE DIFFERENT INDIRECT METHODS.

A. Meyer [2], R. Müller [2], T. Brühl [2], M. Hoffmann [2], J. Bartz [2], G. Hoffmann [3], I. Stavchanska [1], G. Danzl [1], S. Zenk [1]
[1] *SYNLAB Medizinisches Versorgungszentrum Augsburg GmbH, Germany*
[2] *Abbott GmbH, Wiesbaden, Germany*
[3] *Grafrath, Germany*

BACKGROUND-AIM

Accurate reference intervals are crucial for the correct diagnosis of patients. A fast and cost-effective alternative to direct reference interval estimation is provided by indirect methods using routine laboratory data. In the context of transferring thyroid hormone testing from an Abbott ARCHITECT to Alinity i platform, we used three indirect methods to estimate reference intervals for TSH, fT3, and fT4, to assess for potential changes of the currently used reference limits and to verify reference intervals provided by the manufacturer.

METHODS

Routine laboratory data for fT3, fT4, and TSH on Alinity i from two independent laboratories, one in Hamburg, the other in Augsburg (both in Germany) were analyzed. The datasets were cleaned and data pre-processing steps were applied. A modified automated Hoffmann method (RefLim), the Truncated Maximum Likelihood method (TML), and the refineR algorithm were used to calculate reference limits from more than 25.000 and 100.000 individual results for fT3/fT4 and TSH, respectively. Prior to a summarizing analysis data was checked for regional differences and afterwards combined for a final evaluation.

RESULTS

The estimated reference intervals for fT3 were 2.21-3.62 pg/mL for RefLim, 2.30-3.70 pg/mL for TML, and 2.27-3.69 pg/mL for RefineR. For fT4 reference intervals were 0.70-1.13 ng/dL, 0.69-1.21 ng/dL, and 0.73-1.15 ng/dL for RefLim, TML, and RefineR, respectively. For TSH the reference intervals were 0.57-4.00 mIU/L for RefLim, 0.43-3.27 mIU/L for TML, and 0.52-3.7 mIU/L for refineR.
Compared to the existing and manufacturer's representative reference intervals we found some differences, i.e., for the lower limit for fT3, the upper limit for fT4, and both limits for TSH.

CONCLUSIONS

The indirect methods used in the study showed good agreement for fT3 and fT4, whereas for TSH some differences were observed.
Compared to currently used reference intervals, we found some differences which might be attributed to method-specific or regional differences.
As compared to direct reference limit procedures the indirect method used in this study allowed a comparatively easy and time efficient estimation of reference intervals based on already existing data without the need to recruit a distinct reference population.