Reference intervals of the sex hormonal profile in healthy women: A retrospective single-center study in Peru

Jeel Moya-Salazar a, b, *, Sandra P. Cerda c, d, Betsy Cañari e, Marcia M. Moya-Salazar f, Hans Contreras-Pulache g

Graduate School, Universidad Norbert Wiener, Lima, Peru
Department of Pathology, Hospital Nacional Docente Madre Niño San Bartolomé, Lima, Peru
Service of Clinical Laboratory, Salta Lab, Lima, Peru
School of Medical Technologist, Faculties of Health Science, Universidad Norbert Wiener, Lima, Peru
School of Medicine, Faculties of Health Science, Universidad Norbert Wiener, Lima, Peru
Faculties of Pharmacy and Biochemistry, Universidad Norbert Wiener, Lima, Peru
South American Center for Research in Education and Public Health, Universidad Norbert Wiener, Lima, Peru

ARTICLE INFO

Keywords:
Reference intervals
Sex hormones
Lutein hormone
Progesterone
Estrogen
Folic stimulant hormone
Prolactin
Chemiluminescence
Peru

ABSTRACT

The female hormonal profile is of utmost importance for the assessment of the endocrinological functional status and the diagnosis of diseases. The analysis must delimit their normality intervals based on the manufacturer's cut-off points. Due to not all intervals can be evaluated before use, it is imperative to verify the reference intervals to achieve uniformity in the interpretation of results in the female population. We determine the reference intervals of five female sex hormones [Follicle Stimulating hormone (FSH), Estradiol, Luteinizing Hormone (LH), Prolactin, and progesterone] using electrochemiluminescence in the Cobas e411 (Roche). We included female patients > 18 years old, between the 3rd and 15th day of the menstrual cycle (follicular phase) and had no previous medical history or recent medication. For reference intervals analysis, we followed the recommendations of the CLSI C28-A3 guideline. The average concentration for FSH, progesterone, LH, prolactin and estradiol were 11.48 ± 21.10 mIU/ml, 8.19 ± 11.90 ng/ml, 10.98 ± 11.55 ng/ml, 25.05 ± 32.74 ng/mL, and 147.08 ± 473.8 pmol/mL, respectively. Eighty per cent of parameters showed a satisfactory transfer for the manufacturer's reference intervals, except for estradiol, which had 85.5% of transferred values. Our results suggest that 4/5 sex hormones were found within the manufacturer's reference intervals and can be quantified in Peruvian women, ensuring the quality of their results. However, it is necessary to determine the estradiol with other reagents and assays since we show errors in the transfer of intervals.

1. Introduction

The clinical laboratory is a crucial complement to medical decision-making in clinical practice, so serum markers permit the establishment of a sequence of pathophysiological phenomena that ultimately affect the population. Clinical biochemistry is a paramount technique for determining whether circulating female hormones are normal or abnormal in the female population. These assessments allow the interpretations of pathologic events in the endocrine disorders and illnesses, such as diabetes, sex hormone changes, thyroid disorders, among others [1, 2].

One of the most frequently changes in the adult population is the imbalances of sexual hormones that lead to the loss of libido, behavioural changes, infertility, and other endocrine syndromes such as hypothyroidism [3]. An accurate understanding of these alterations, treating and controlling possible significant imbalances in the feminine population, is vital in obtaining confidence results and very close to reality. It can undoubtedly be achieved by ensuring the quality of the results, as an error in quality management can lead to a misinterpretation of results triggering medically relevant affects in the treatment and health of women [4, 5].

To provide high-quality results, clinical laboratories must establish a quality management process to ensure reliable results [6]. One of the principal quality assurance processes is the timely evaluation of reference intervals that commercial houses attribute to the reagents to interpret the estimated values in the user population and appreciate their normality [7, 8].

* Corresponding author.
E-mail address: jeel.moya@uwiener.edu.pe (J. Moya-Salazar).

https://doi.org/10.1016/j.heliyon.2022.e10592
Received 1 July 2021; Received in revised form 22 January 2022; Accepted 5 September 2022
2405-8440/ © 2022 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
As per ISO 15189:2012, the Clinical and Laboratory Standard Institute (CLSI), and the Clinical Chemistry International Federation (IFCC), these intervals must be verifiable periodically and demandingly when new analysis tests are incorporated into a population that has not gotten previous quality evaluations. Therefore, clinical decision values have not been correctly positioned [9, 10]. Since the 1960s, verification processes and transfer of trade reference ranges for several markers in clinical chemistry, immunology, and haematology have been used with greater eagerness and uniformity [7, 8, 11, 12, 13, 14]. Nevertheless, many countries have not yet regulated the reference intervals analysis for all the markers; for example, in Peru, the thyroid or feminine hormone profiles have not yet been verified despite their constant demand.

The knowledge of commercial reference intervals could be transferable or not to the Peruvian population. It will provide consistency in the clinical interpretation of hormonal analysis results, ensuring the quality of medical attention to patients who present changes in their hormonal levels, especially in this context of the government’s promotion of quality and the decedence of clinical analysis offered by Peruvian laboratories [15].

The objective of this study was to determine the reference values of female hormonal profiles in healthy adults in Peru.

2. Methods

2.1. Study design and location

A retrospective single-centre study was conducted in 2019. Patient selection and hormone profile analysis were performed in the Suiza Lab in Miraflores, Lima (Peru). This laboratory carries out clinical analysis of hospitals, clinics, and research centres nationwide. It also has a comprehensive quality system supervised every semester by the American College of Pathologists.

2.2. Samples, volunteers and inclusion criteria

All patients enrolled voluntarily in this study, with previous approval and after filling up the informed consent form. We included female patients >18 years of age, between the 3rd and 15th day of the menstrual cycle (follicular phase) and had no previous medical history or recent medication. We excluded pregnant women, patients with neoplasms, cardiovascular and autoimmune diseases, chromosomopathies, anabolic consumption, hormonal therapy for infertility, and those with a personal and family history of the endocrinological disease.

2.3. Hormonal assessment

The collection of samples has been performed by venipuncture with the BD Vacutainer system (Leax Point, France) according to the specific date for the sampling following the Standard Operational Process (SOP) of the laboratory. Samples were processed with the electrochemiluminescence method in the automated system of the fourth generation of Cobas e411 (Roche Diagnostics, Chicago, USA). The female hormonal profile included the simultaneous determination of Stimulating Follicle (FSH), Luteinizing Hormone (LH), Estradiol (E2), progesterone, and prolactin.

Three levels of quality control materials provided by the manufacturer (PreciControl ISD from Roche Diagnostics) were used to check the precision of the study. The results of these controls were measured double daily, within the established threshold (10%) and monthly reported to the CAP external quality assessment (EQA) program. Within this programme, the analytical sensitivity of each marker and performance measures (i.e., the limit of quantification) were also verified using CAP EQA materials and acceptance criteria that laboratory met monthly. This hormonal evaluation system participates in Roche’s external quality control program, which ensures the traceability of the results, as well as internal control.

2.4. Reference intervals and data analysis

The analysis results were codified directly into the data matrix in IBM SPSS v24.0 (Armonk, US) for macOS. After patients were enrolled in the study, their clinical criteria were reviewed in their medical record to consider them as part of the sample. All those who did not meet the requirements were discarded. After this, the normality of the results with the Kolgomorov-Smirnov test was evaluated.

Then, following the recommendations of the CLSI C28-A3 guideline [16], a descriptive analysis and a reference interval analysis were developed. This analysis included the detection of marginal values with the Tukey y Dixon- Reed method [17, 18] considering a significance threshold of 5% and excluding outliers that were outside three standard deviations of the mean or had extreme values at or outside the normal limit. Estimation of the lower reference limit (2.5% percentiles) and the upper reference limit (97.5% percentiles), the comparison of obtained intervals and estimation of transfer of values in front of commercial intervals for each test of the female hormonal profile, considering a cut-off of 95% for successful transfer. For all analyses, the p-value < 0.05 and a 95% confidence interval (CI) were considered significant. Finally, we used BoxPlotR (Tyers and Rappsilber labs, Berlin) for figure designs.

2.5. Ethical aspects

This study had the approval of the Laboratory Management (Nº SL-O1-046-2019) and for the Ethics Research Committee of the Universidad Norbert Wiener (UNW-VRI-Nº 054-2019, Date: Jan 15th, 2019).

3. Results

3.1. Participants

We included a total of 707 patients who met the inclusion criteria, but 48 patients were excluded due to extreme values. In the analysis of 659 samples included in the study, the normal distribution of all-female markers was determined (all p > 0.05). The average age of the participants was 29.9 ± 6.1 years (range 18–40 years) and all the women were from Lima, Peru. Figure 1A–E shows the distribution of each hormonal marker.

3.2. Main results

Amongst the hormonal markers, progesterone was the one with the highest number of analyses (n = 156). The average concentration of FSH was 11.48 ± 21.1 mlU/mL (95% CI, 7.9 to 15.1) and LH was 10.58 ± 11.6 ng/mL (95% CI, 9.1 to 12.9) (Figure 2). While the average serum concentration of progesterone, prolactin and estradiol was 8.19 ± 11.9 ng/mL (95% CI, 6.3 to 10.1), 24.29 ± 32.7 ng/mL (95% CI, 19.5 to 30.6), and 147.1 ± 473.8 ng/mL (95% CI 66.3 to 227.9), respectively (Table 1).

Four of the five markers of hormonal function showed 100% of their values in the manufacturer’s reference intervals. Only estradiol did not have a satisfactory verification or transfer of normal values in the Peruvian population, with an average of 85.5% with the electrochemiluminescence method. In Table 2, we show the validation and transfer of the estimated reference interval in the study population.

4. Discussion

This is the first study in Peru to assess the status of female hormones and found that 100% of the data on the four sex hormones (FSH, LH, progesterone, and prolactin) of women of childbearing age have been transferred. Estradiol did not have a successful transfer when analyzed in Cobas e411 with the electrochemiluminescence method. Even if the hormonal parameter depends on a large number of factors that include the technique and the reagents used for its determination, the preanalytic phase of the study, as well as the adequacy of the sample,
lack of evaluation, unrecognized and little investment in quality assurance are critical factors [19, 20]. A recent study [21] determined that the specific reference range for estrogen (E1) and estradiol (E2) in women of childbearing age is 3–12 pmol. Our findings are inconsistent with this study because our interval is much longer (5.0–2603.4 pmol/L). These marked differences could be attributed to changes in the analysis methods because this study was related to liquid chromatography-mass spectrometry (LC-MS/MS), which is more sensitive than

Figure 1. Histograms and distribution of female sex hormones. Stimulating Follicle Stimulating hormone (A), Estradiol (B), Luteinizing Hormone (C), Prolactin (D) and progesterone (E).

Figure 2. Distribution of the analysis of sex hormones in Peruvian women.
Our results revealed a significant increase with age while prolactin decreased [30]. For their part, Schüring et al. have demonstrated that the reference interval of prolactin is affected by hormonal status in 139 reproductive women and 103 postmenopausal women [12]. Both studies allow us to understand the importance of the choice of the study population and the monitoring of patients. In this study, the monitoring of the volunteers was not according to the menstrual cycle. Our results have demonstrated the verification and transparency of reference intervals of the five female hormones in Peru. These results cannot be generalized due to possible fluctuations in the values during the menstrual cycle. We prove that the intervals for FSH, LH, progesterone, and prolactin were satisfactorily transferred and can be applied as general ranges in the Peruvian population in the absence of other reports.

The limitations of the study were 1) the study developed an evaluation in a single-centre, being necessary to carry out multicenter studies considering multiculturalism and Peruvian biological variability; 2) we verify the reference intervals on the Cobas e411 electrochemiluminescence analyzer. However, it is necessary to evaluate the transfer of intervals in other platforms widely used in Peru such as Enzyme-Linked ImmunoSorbent Assay, chemiluminescence, and LC-MS/MS, finally 3) the intervals of reference were verified for fertile women population, further studies are necessary to evaluate the analysis of women of menopausal, pre-menopausal age, pre-pubertal stage, in replacement therapy, transgender community, men and child population.

In conclusion, this study shows the verification of 4/5 parameters of the female hormonal profile evaluated in the Peruvian population of fertile age in the follicular phase. Estradiol transfer to the manufacturer’s reference intervals defined in the study using the electrochemiluminescence method was unsuccessful.

It is the first verification study of the reference intervals of sexual hormones in Peruvian women. In further studies, we suggest that it is necessary to continue qualitative research to understand changes in hormonal intervals and design a consensus report on clinical use.

### Declarations

**Author contribution statement**

Jeel Moya-Salazar, Sandra P. Cerda: Conceived and designed the experiments, performed the experiments, analyzed and interpreted the data, contributed reagents, materials, analysis tools or data, wrote the paper.

Marcia M. Moya-Salazar: Conceived and designed the experiments, performed the experiments, contributed reagents, materials, analysis tools or data, wrote the paper.

Hans Contreras-Pulache: Conceived and designed the experiments, analyzed and interpreted the data, wrote the paper.

Betsy Cañari: Performed the experiments, analyzed and interpreted the data, wrote the paper.

**Funding statement**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

---

**Table 1. Baseline characteristics of the five female sexual markers of Peruvian patients.**

| Sex hormones | N   | X ± DS | Range          | 95% CI          | p-value* |
|--------------|-----|--------|----------------|----------------|----------|
| FSH (mU/mL)  | 131 | 11.48 ± 21.10 | 0.33-162.8 | 7.89-15.08 | 0.072 |
| Progesterone (ng/mL) | 155 | 8.19 ± 11.90 | 0.05-60 | 6.31-10.07 | 0.095 |
| LH (ng/mL)   | 121 | 10.58 ± 11.55 | 0.1-63.85 | 9.01-12.95 | 0.857 |
| Prolactine (ng/mL) | 120 | 24.29 ± 32.74 | 0.26-339.8 | 19.46-30.63 | 0.094 |
| Estradiol (ng/mL) | 131 | 147.08 ± 473.8 | 5-500 | 66.25-227.92 | 0.077 |

* Results of Kolgomorov-Smirnov test. Abbreviation: FSH: Stimulating Follicle Hormone, LH: Luteinizing Hormone.

**Table 2. Comparison between study range and manufacturer’s intervals for five female sex markers.**

| Sex hormones | N   | Study intervals (2.5%–97.5%) | MRI | Transfer (%) | Verification |
|--------------|-----|-----------------------------|-----|--------------|--------------|
| FSH (mU/mL)  | 131 | 1.30-96.70                  | 0.100-200 | 100 Yes | | 2.5-97.5% |
| Progesterone (ng/mL) | 156 | 0.01-58.20                | 0.05-60 | 100 Yes | | |
| LH (ng/mL)   | 121 | 0.30-52.0                   | 0.100-200 | 100 Yes | | |
| Prolactine (ng/mL) | 120 | 3.30-92.10                | 0.0470-470 | 100 Yes | | |
| Estradiol (pmol/mL) | 131 | 5.00-2603.4                | 18.4-11010 | 85.5 No | | 2.5-97.5% |

Abbreviation: FSH: Stimulating Follicle Hormone, MRI: Manufacturer reference intervals, LH: Luteinizing Hormone.

...
Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] R.C. Austin, T.J. Bussey, K.L. Cortes, Biochemistry education: from theory to practice, in: ACS Symposium Series 1337, American Chemical Society Press, Washington D.C., 2019.
[2] J. Berg, The approach to pathology harmony in the UK, Ann. Clin. Biochem. 33 (3) (2012) 89-95.
[3] M. Hausmann, Why sex hormones matter for neuroscience: a very short review on sex, sex hormones, and functional brain asymmetries, J. Neurosci. Res. 95 (1-2) (2017) 40–49.
[4] K.M. Aakre, M.R. Langlois, J. Watine, J.H. Barth, H. Baum, P. Collinson, et al., Critical review of laboratory investigations in clinical practice guidelines: proposals for the description of investigation, Clin. Chem. Lab. Med. 51 (6) (2013) 1217–1226.
[5] N.J. Schork, A.B. Weder, M.A. Schork, On the asymmetry of biological frequency distributions, Genet. Epidemiol. 7 (1990) 427–446.
[6] J.O. Westgard, Design of internal quality control for reference value studies, Clin. Chem. Lab. Med. 42 (7) (2004) 863–867.
[7] C.Y. Lazo, P.A. López, Verificación de intervalos de referencia de analitos más frecuentes en el área de Química Clínica en el laboratorio del Centro Médico Naval, (Thesis) Lima, Facultad de Medicina, Universidad Peruana Cayetano Heredia, 2018.
[8] S.J. Marrero, C.R. Lárez, Y.M. Avilés, J.A. Segovia, A.Y. Chirinos, M.A. Romero, et al., Verificación y transferencia de intervalos de referencia del perfil tiroideo y PSA total en individuos masculinos de la ciudad de Valencia, Venezuela, Rev. Latinoam. Patol. Clin. Med. Lab. 64 (2) (2017) 94–99.
[9] F. Schneider, C. Maurer, R.C. Friedberg, International organization for standardization (ISO) 15189, Ann. Lab. Med. 37 (5) (2017) 365–370.
[10] International Federation of Clinical Chemistry, Defining, establishing, and verifying reference intervals in the clinical laboratory, in: Approved Guideline, third ed., C28-A3, Clinical and Laboratory Standards Institute, Wayne, PA, 2008.
[11] M. Ayetekin, K. Emerk, Accurate reference intervals are required for accurate diagnosis and monitoring of patients, eJIFCC 19 (2) (2008) 1–5.
[12] A.S. Digheea, J.H. Moya, F.J. Hayes, P.M. Sluss, High-resolution reference ranges for estradiol, luteinizing hormone, and follicle-stimulating hormone in men and women using the AxSYM assay system, Clin. Biochem. 38 (2005) 175–179.
[13] D.N. Greene, R.L. Schmidt, G.W. McPherson, J. Ronginsch, K.L. Imborek, J.A. Dickerson, et al., Reproductive endocrinology reference intervals for transgender women on stable hormone therapy, J. Appl. Lab. Med. 6 (1) (2021) 15–26.
[14] A.N. Schüring, R. Kelch, G. Pierczyński, J.R. Nofer, Establishing reference intervals for sex hormones on the analytical platforms advia centaur and immulite 2000XP, Ann. Lab. Med. 36 (1) (2016) 55–59.
[15] J. Moya-Salazar, L. Pio-Davila, Evaluation of inter-batch variability in establishing and quality control of glucose, Med. Univ. 18 (71) (2016) 85–90.
[16] Clinical and Laboratory Standards Institute, Defining Establishing and Verifying Reference Intervals in Clinical Laboratory; Approved Guidelines, third ed., CLSI document C28-A3, Wayne, PA., 2008.
[17] A.H. Reed, R.J. Henry, W.B. Mason, Influence of statistical method used on the resulting estimate of normal range, Clin. Chem. 7 (4) (1971) 275–284.
[18] J.W. Tyuey, Exploratory Data Analysis, Addison-Wesley, Reading MA, 1997.
[19] A.R. Haddad, D. Giacherio, A.L. Barkan, Interpretation of common endocrine laboratory tests: technical pitfalls, their mechanisms and practical considerations, Clin. Diabetes Endocrinol. 5 (2019) 12.
[20] H.W. Vesper, J.C. Botelho, Y. Wang, Challenges and improvements in testosterone and estradiol testing, Asian J. Androl. 16 (2) (2014) 178–184.
[21] H. Frederiksen, T.H. Johannsen, S.E. Andersen, J. Albrethsen, S.K. Landersoe, J.H. Petersen, et al., Sex-specific estrogen levels and reference intervals from infancy to late adulthood determined by LC-MS/MS, J. Clin. Endocrinol. Metab. 105 (3) (2020) dg1196.
[22] S.J. Virdonk, H.W. Vesper, F.D. Martens, P.M. Sluss, J.J. Hillebrand, A.C. Heijboer, Estradiol reference intervals in women during the menstrual cycle, postmenopausal women and men using an LC-MS/MS method, Clin. Chim. Acta 495 (2019) 198–204.
[23] A. Radicioni, A. Lenzi, M. Spaziari, A. Anzini, G. Ruga, G. Papi, et al., A multicenter evaluation of immunoaassays for follicle-stimulating hormone, luteinizing hormone and testosterone: concordance, imprecision and reference values, J. Endocrinol. Invest. 36 (2013) 739–744.
[24] B. Bogner, K. Schwenoba, M. Vogt, D. Weghuber, C. Roth, U. Kipman, et al., Evaluation of reference intervals of haematological and biochemical markers in an Austrian adolescent study cohort, Clin. Chem. Lab. Med. 57 (6) (2019) 891–900.
[25] L. Mewton, K. Champion, F. Kay-Lambkin, M. Sunderland, L. Thornton, M. Teesson, Lifestyle risk indices in adolescence and their relationships to adolescent disease burden: findings from an Australian national survey, BMC Publ. Health 19 (2019) 60.
[26] M. Al-Chalabi, A.N. Bass, I. Alsulaiman, Physiology, prolactin, in: StatPears Treasure Island, StatPears Publishing, 2021.
[27] K.D. Lahiri, M. Baruah, J. Ghosh, S. Sengupta, Establishment of reference interval of total triiodothyronine (TT3) and thyrotropin (TSH) and quality control of glucose, Med. Univ. 18 (71) (2016) 85–90.
[28] S.J. Whitehead, M.P. Cornes, C. Ford, R. Gama, Reference ranges for serum total oestradiol, luteinizing hormone, and follicle-stimulating hormone in men and women using the AxSYM assay system, Clin. Biochem. 38 (2005) 175–179.
[29] M. Overgaard, S.M. Pedersen, Serum prolactin revisited: parametric reference values, J. Clin. Diagn. Res. 8 (7) (2014) 1226–1229.
[30] A.R. Haddad, D. Giacherio, A.L. Barkan, Interpretation of common endocrine laboratory tests: technical pitfalls, their mechanisms and practical considerations, Clin. Diabetes Endocrinol. 5 (2019) 12.
[31] H.W. Vesper, J.C. Botelho, Y. Wang, Challenges and improvements in testosterone and estradiol testing, Asian J. Androl. 16 (2) (2014) 178–184.
[32] H. Frederiksen, T.H. Johannsen, S.E. Andersen, J. Albrethsen, S.K. Landersoe, J.H. Petersen, et al., Sex-specific estrogen levels and reference intervals from infancy to late adulthood determined by LC-MS/MS, J. Clin. Endocrinol. Metab. 105 (3) (2020) dg1196.
[33] S.J. Virdonk, H.W. Vesper, F.D. Martens, P.M. Sluss, J.J. Hillebrand, A.C. Heijboer, Estradiol reference intervals in women during the menstrual cycle, postmenopausal women and men using an LC-MS/MS method, Clin. Chim. Acta 495 (2019) 198–204.
[34] A. Radicioni, A. Lenzi, M. Spaziari, A. Anzini, G. Ruga, G. Papi, et al., A multicenter evaluation of immunoaassays for follicle-stimulating hormone, luteinizing hormone and testosterone: concordance, imprecision and reference values, J. Endocrinol. Invest. 36 (2013) 739–744.
[35] B. Bogner, K. Schwenoba, M. Vogt, D. Weghuber, C. Roth, U. Kipman, et al., Evaluation of reference intervals of haematological and biochemical markers in an Austrian adolescent study cohort, Clin. Chem. Lab. Med. 57 (6) (2019) 891–900.
[36] L. Mewton, K. Champion, F. Kay-Lambkin, M. Sunderland, L. Thornton, M. Teesson, Lifestyle risk indices in adolescence and their relationships to adolescent disease burden: findings from an Australian national survey, BMC Publ. Health 19 (2019) 60.
[37] M. Al-Chalabi, A.N. Bass, I. Alsulaiman, Physiology, prolactin, in: StatPears Treasure Island, StatPears Publishing, 2021.
[38] K.D. Lahiri, M. Baruah, J. Ghosh, S. Sengupta, Establishment of reference interval of total triiodothyronine (TT3) and thyrotropin (TSH) and quality control of glucose, Med. Univ. 18 (71) (2016) 85–90.
[39] S.J. Whitehead, M.P. Cornes, C. Ford, R. Gama, Reference ranges for serum total oestradiol, luteinizing hormone, and follicle-stimulating hormone in men and women using the AxSYM assay system, Clin. Biochem. 38 (2005) 175–179.
[40] M. Overgaard, S.M. Pedersen, Serum prolactin revisited: parametric reference intervals and crossplatform evaluation of polyethylene glycol precipitation-based methods for discrimination between hyperprolactinemia and macroprolactinemia, Clin. Chem. Lab. Med. 55 (11) (2017) 1744–1753.