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Original Paper

VARIATION OF SERUM VITAMIN B12 LEVEL IN THE BLOOD FROM PATIENTS OF DIFFERENT AGE AMONG THE LATVIAN POPULATION

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The reference intervals and median values of serum vitamin B12 level in blood from patients among the Latvian population were established for different age groups with a two-year step using data for a large number of patients, accumulated in E. Gulbis Laboratory in Latvia. The data represents the general population of Latvia. An indirect in silico method, developed in E. Gulbis laboratory for determination of serum vitamin B12 blood level reference intervals, was used. Strong elevation of serum vitamin B12 blood level was observed in children who were between 2 and 10 years old. The paper discusses the serum vitamin B12 values for children and a different serum vitamin B12 reference interval for young children is suggested.

Keywords: *B12 vitamin, reference interval, large patient data, children,* in silico *analysis, general population, Latvia.*

INTRODUCTION

An accurate reference interval (RI) plays an important role in day-to-day medical practice as a guide for interpreting the results of clinical analysis. For serum vitamin B12, there are several studies proposing RIs for serum vitamin B12, the most recent of which was Gavars *et al*. (2022). RI usually takes the central 95% of the result distribution in a reference population for a clinical test. There are two ways for establishing RIs — direct and indirect methods. The direct method is by experimentally establishing the RI from recruited and preselected healthy individuals, which is rather time consuming and costly. This is one of the reasons why usually there is just one serum vitamin B12 RI for the whole population. In the direct method, a number of healthy individuals are recruited and tested for the sole reason to establish the RI. The minimal number of reference individuals is 120 (90% confidence limit) (Horowitz *et al*., 2010). If the desired confidence limit needs to be higher, a larger number of reference individuals should be selected. In practice, a higher amount of reference individuals might be needed to reach the desired level of accuracy and confidence level.

In the indirect method there is no recruitment of individuals for the purpose of establishing the RI; the method instead uses patient data from laboratories, which have been acquired during a certain time period. The data, usually from a large number of different patients, are far greater than the minimum of 120 patients required for the direct method, and are mathematically manipulated to transform the patient density distribution to Gaussian or some other commonly used distribution to enable the extraction of the RIs. Variations of the indirect method have options for patient selection, by identifying healthy individuals for further RI calculation. Owing to the advance of computer technology and the availability of large amounts of clinical test data, the indirect method on how to establish RIs using data accumulated in laboratories, medical establishments or elsewhere

has been used and become available and affordable. The idea behind the indirect method is to use the accumulated test data and assume that there is a sufficient number of patients, who can be considered "healthy" and "normal" in the selected clinical test and then use various mathematical methods to separate the part of the patient density distribution that corresponds to the "normal" group of patients. The most recent description of the different indirect methods is provided by Ozarda *et al.* (2021).

By using the indirect method, the initial patient data can be screened to exclude patients with abnormal results that show up in other clinical tests. Several mathematical methods can then be applied to extract the patient density distribution function and the RIs are then calculated from the part of the patient density distribution function that corresponds to "normal" patients. A Box-Cox transformation (Box and Cox, 1964) can be applied to the initial patient density distribution function to make it more similar to Gaussian or some other distribution that allows for calculation of standard deviations. In some cases, the distribution function is decomposed as a sum of several Gaussian distributions with one corresponding to "normal" patients, while the other cases have only a part of the patient distribution corresponding to "normal" patients used in calculating the standard deviation of the distribution function. The initial data set can be truncated in order to select the region that best corresponds to "normal" patients (Arzideh *et al.*, 2007; Wosniok and Haeckel, 2019).

Indirect methods have the advantage of having a large number of patients that can be used for calculating the RIs, as well as its cost per patient being very low. This allows for test RIs of different subgroups such as age, sex and ethnicity, where otherwise it would be very difficult and expensive to recruit enough healthy individuals for each subgroup. The use of indirect methods in establishing RI requires careful analysis of the data in order to be able to test the underlying assumptions about the cohort of patients used in the calculations and the patient distribution functions.

Apart from our previous paper (Gavars *et al.*, 2022) we have not identified any publication where the RI for serum vitamin B12 has been established by indirect methods. Some recent studies have reported serum vitamin B12 RIs for different population groups – comparing between Chinese and European populations (Jiang *et al.*, 2020), as well as between different ethnicities in the United Kingdom (O'Logbon *et al.*, 2021). In the case of serum vitamin B12 blood concentration, the determination of adequate RI is further complicated due to overlapping nutritional impact, where studies in Nepal and India (Ng'Eno *et al.*, 2017; Singh *et al.*, 2017) have shown considerably lower average serum vitamin B12 values than in western countries.

Some studies have reported a serum vitamin B12 RI for different age groups; however, the results show a large dispersion between different studies. In Figure 1 the median value of serum vitamin B12 concentration for different age groups

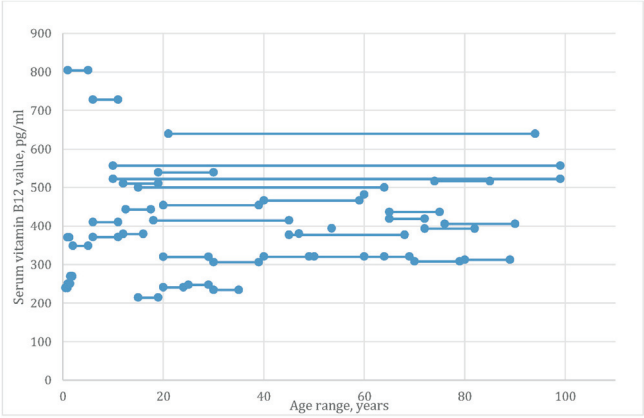

Fig. 1. Median serum vitamin B12 levels from the literature (Hao *et al.*, 2007; Gilsing *et al.*, 2010; González-Gross *et al.*, 2012; Henry *et al.*, 2012; Cobayashi *et al.*, 2015; Risch *et al.*, 2015; Orton *et al.*, 2016; Ng'Eno *et al.*, 2017; Singh *et al.*, 2017; Devi *et al.*, 2018; Kim *et al.*, 2018; Mathias *et al.*, 2018; Silva *et al.*, 2019; Esnafoglu and Ozturan, 2020; Flores-Guerrero *et al.*, 2020; Al-Musharaf *et al.*, 2021; Miki *et al.*, 2021; Abildgaard *et al.*, 2022; Disease Control and Center for Environmental Health, n.d.,).

is presented. Since the experimental uncertainty in serum vitamin B12 measurements usually is at least 20% (Referenzinstitut für Bioanalytik, 2020), most results, including on children, are consistent. Most often the level of B12 has been studied due to an existing diagnosis in unspecified sex and age groups.

One of the most comprehensive studies for different age groups was published by the Disease Control & Center for Environmental Health, n.d.), where the serum vitamin B12 blood values were reported for different age groups in the USA. In this report, significantly elevated serum vitamin B12 values are reported for children aged between one to five years and between six and eleven years. In other reports, considerably lower serum vitamin B12 values for children have been reported for Nepal (Ng'Eno *et al.*, 2017), Brazil (Cobayashi *et al.*, 2015), and India (Singh *et al.*, 2017), and a significant part of people studied were reported to be with some form of B12 deficiency that was linked to low intake of serum vitamin B12. In Abildgaard *et al.* (2022) serum vitamin B12 and methylmalonic acid levels (a marker of serum vitamin B12 physiological deficiency) were studied in the ethnic Danish population of Denmark, with the aim of establishing RIs for different age groups. In this study, elevated serum vitamin B12 RIs were suggested for children up to 11 years old. The results of Abildgaard *et al.* (2022) also included rounded up and locally verified results from a large study of serum vitamin B12 levels conducted by the Canadian Laboratory Initiative on Paediatric Reference Intervals (CALIPER) (Bohn, 2019). In the CALIPER study, serum samples from 741 healthy Canadian children from the Greater Toronto Area and Hamilton regions aged between 1 day and 18 years were measured.

While median serum vitamin B12 values reported in the literature are quite dispersed the serum vitamin B12 defi-

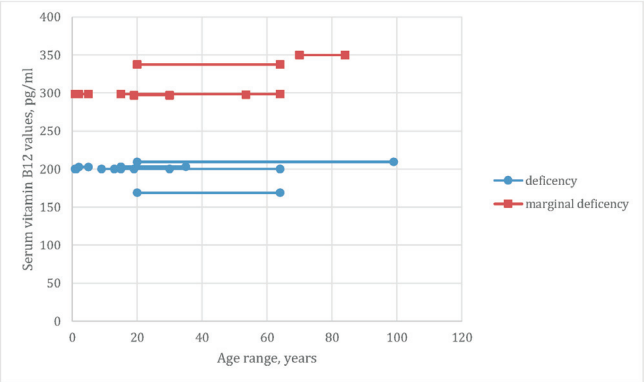

Fig. 2. Serum vitamin B12 deficiency levels as defined in the literature. Blue circles are values reported as "deficiency" and orange squares are values reported as "marginal deficiency" (Cobayashi *et al*., 2015*;* Orton *et al*., 2016; Singh *et al*., 2017*;* Devi *et al*., 2018*;* Kim *et al*., 2018*;* Silva *et al*., 2019*;* Flores-Guerrero *et al*., 2020; Soh and Won, 2020; Al-Musharaf *et al*., 2021*;* Fuzo *et al*., 2021).

ciency values found in the literature are defined more uniformly, as can be seen in Figure 2.

B12 vitamin is not synthesised by human cells, it is sourced from food, or in forms of pills and injections (Fatima *et al*., 2023), although a small amount of B12 vitamin can be produced by the gut microflora. The level of B12 level in the blood thus depends on the health conditions, lifestyle, as well as the dietary patterns. There are reports linking the B12 blood level to alcohol consumption (Laufer *et al*., 2004; Parackal *et al*., 2020; Broz *et al*., 2022).

Manufacturers of serum vitamin B12 tests usually report just one RI value for all age groups. Since the available research results indicate that different populations in different countries and within different age groups have different serum vitamin B12 values, it is interesting and important to investigate the serum vitamin B12 blood level in more detail for the Latvian population.

In this work, the RI and median serum vitamin B12 values for different age groups with a two-year age range for the Latvian population were determined using the indirect method.

MATERIALS AND METHODS

Patient selection. We used data from all patients that were given a B12 test in E. Gulbis laboratory within the time interval starting from the beginning of 2018 until 21 November 2021. In total, B12 test data from 119995 patients were used in this study. The patients were from Latvia, from all parts of the country. The patient composition was deemed to be representative of the average population of Latvia and consisted of 62.99% Latvian, 24.22% ethnic Russian, 2.23% Ukrainian with a negligible fraction of Black, Hispanic, Asian, and other non-European population groups according to the national census (Iedzīvotāji pēc tautības…, n.d.). All types of patients were used in the study, which included patients from hospitals, patients directed by their physicians and patients who individually on their own initiative decided to test their serum vitamin B12 level. Both patients with their tests paid for by the state health care system and those who were paying themselves for the tests were included in the study. No patients or groups of patients were excluded from the study — all the serum vitamin B12 tests performed within the period stated above were included in the study.

Laboratory methods. All serum vitamin B12 blood level tests were performed at the E. Gulbis Laboratory (ISO/IEC 17025, ISO 15189:2013) in Rīga, Latvia. E. Gulbis Laboratory is a clinical laboratory performing clinical tests for all regions of Latvia and for all types of patients.

Electrochemiluminescence immunoassays (ECLIA) (Roche Diagnostics, 2010) performed on the Cobas e 801 analySer was the method used. The manufacturer's RI for this method is set at 197–771 pg/ml (146–569 pmol/l), which was similar for all ages. The measuring range is 100–2000 pg/ml or 73.8–1476 pmol/l (Allen, 2012).

According to the laboratory procedure, the accuracy of the serum vitamin B12 tests was regularly checked against standardised samples. The coefficient of variation (CV), defined as the standard deviation (SD) divided by the mean and multiplied by 100, was calculated. The target CV was 9.5%, but typically the CV was below 4%. The CV was calculated from daily internal quality control results. The control material was the same every day, so a lower CV value is better. The target value for the coefficient of variation (CV) means that if the coefficient of variation is lower than the target value of 9.5%, then E. Gulbis laboratory accepts the results and does not perform corrective actions. Usually, the CV in E. Gulbis laboratory was lower than the target value. E. Gulbis Laboratory has informed us that the CV typically is 4% which means that the data series have less dispersion and thus the data are better. The CV between different laboratories within the framework of international quality control is 15% (Referenzinstitut für Bioanalytik, 2020).

Statistical methods. In our work, the RI of serum vitamin B12 vitamin blood level and median serum vitamin B12 values for different age groups was experimentally determined from a large number of serum vitamin B12 patient test data accumulated by E. Gulbis Laboratory in Latvia. An indirect method for establishing serum vitamin B12 RI from a large amount of patient data was developed in the E. Gulbis Laboratory (Gavars *et al*., 2022). We transformed original patient density distribution by using Box-Cox transformation (Box and Cox, 1964) with $\lambda = 0$. In our efforts to transform the experimental patient distribution into a Gaussian distribution, we found that the trivial solution of Box-Cox transformation with $\lambda = 0$ gave good transformation of the experimentally observed distribution into a Gaussian distribution and by following the minimalistic principle we used this form of transformation. The patient density distribution was truncated at 30% of all patients, centred around the median value. The selection of some part of the patient distribution to find the Gaussian parameters is used when there are good reasons to assume that there are

parts of the distribution that cannot be transformed into a single Gaussian distribution. We tested various cut-offs around the central part of the distribution and found that after the selection of the central 30% the further cut-offs resulted in the convergence of the calculated Gaussian parameters towards a single value. The 30% cut-off was the best trade-off between having good Gaussian shape and sufficiently high number of experimental data points for the statistics. The parameters that we determined from the distribution after it was transformed by applying Box-Cox transformation were the median value of B12 blood serum level and the RI — that is, the cut-off at 2.5% and 97.5% of the distribution.

The laboratory analyser used for serum vitamin B12 tests divided the whole serum vitamin B12 value range from 100 to 4000 pg/ml into 3902 channels with a constant channel breadth in the linear serum vitamin B12 value scale. In the Box-Cox transformation (Box and Cox, 1964), the serum vitamin B12 values, that is the number of channels, was kept constant and the change in the breadth of the channel was compensated by using a transformation coefficient on the number of patients per channel. For low serum vitamin B12 values close to 100 pg/ml, the number of patients per serum vitamin B12 value channel were not distributed in a statistically uniform way and the same applies for very large values, which were close to 4000 pg/ml. We tested the smoothness of the patient distribution by comparing the actual patient count per channel with the averaged patient count per channel using the Butterworth filter method for smoothing the curve. The mean standard deviation per channel was defined as the square root of the number of patients per channel. There were deviations from the expected standard deviation distribution with significantly increased number of mean standard deviations in the interval starting from 2.9 to 41. To cancel out these deviations in the patient density distribution, the integrated Gaussian distribution was used for the calculations, while to fit the Gaussian parameters in the truncated integrated patient distribution the least square method was used. To test the quality of the fit to the Gaussian distribution of the truncated patient distribution after the Box-Cox transformation (Box and Cox, 1964), the Kolmogorov–Smirnov test was used. With truncation standing at 30% (±15%) around the median and the median correction being 0.7% towards lower serum vitamin B12 values, the Kolmogorov–Smirnov test gave a KS D value of 0.02 and KS p-value of 0.994018, which that corresponds to a very high likelihood that the central part of the distribution is indeed Gaussian.

In total 172 '597 data points from serum vitamin B12 patient analyses were used. This number of data points allowed us to establish serum vitamin B12 RIs with high precision for all age groups with a very small step of just two years.

The median values of serum vitamin B12 for different age groups were calculated by averaging all measurements. The patients with multiple tests were thus overrepresented, however the comparison with results obtained by using different methods, such as selecting just one result for one patient, showed that the influence on the median value was insignificant. The median values and the RI of serum vitamin B12 blood level for each age group were calculated with 2-year steps, apart from the age group 0–2 years where a one-year step was used because of the rapid changes in serum vitamin B12 levels in this age group.

The central assumption of the method used for the calculation of RI from the accumulated data was that most of the patients were "normal" in respect to their serum vitamin B12 blood levels. After the Box-Cox transformation (Box and Cox, 1964), the parameters of the Gaussian patient density distribution from the central part of the experimental data set were calculated. One-third of the patients with serum vitamin B12 blood level around the median value were used for the calculation of the Gaussian patient density distribution. For the Gaussian patient density distribution, the highest and lowest ends of the RI were calculated. This method for truncating the patient density distribution at 30% for the serum vitamin B12 RIs was tested and validated in our previous work (Gavars *et al*., 2022), where it was shown that by progressively selecting patients centred closer and closer to the median serum vitamin B12 value, the calculated serum vitamin B12 RIs converged to the values that correspond to patients labelled as "serum vitamin B12 normal".

RESULTS

The calculated median serum vitamin B12 values together with the calculated lowest and highest end of the RIs are shown in the Figure 3. The number of patients in each age group is shown in the Figure as grey columns. The results showed that children aged 2–10 years had strongly elevated serum vitamin B12 values reaching the mean value of 660 pg/ml for 3–5 years old children, compared to 410 to 480 pg/ml for the rest of the population. For the other age groups there was only slight variation in B12 median values with the age of patients. There was a slight dip for the 15- to 27- year- old age group where the median value wis 410 to 425 pg/ml.

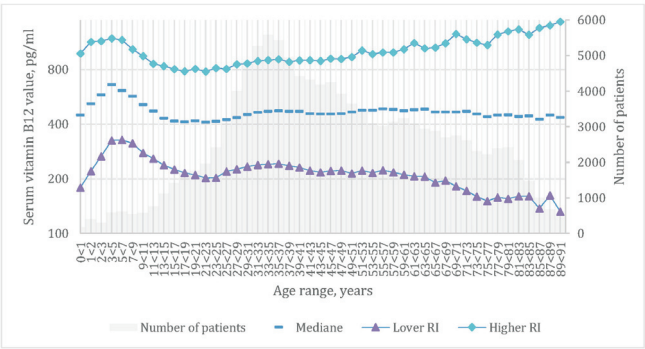

Fig. 3. Median serum vitamin B12 blood level and both low and high end of RI calculated from a large number of patients. The median value is represented by horizontal lines while squares and triangles are calculated low and high ends of RI, respectively.

We did additional calculations and tests to confirm that the increase for children was real and not some effect of patient selection in this age group. As the calculated median values and RIs are based on assumption that most of the patients are "serum vitamin B12 healthy" and "serum vitamin B12 normal", a separate analysis was performed for the children aged 2–10 years to test this assumption. We looked at whether there were indications that in this age group the children tested were predominantly those with medical or nutritional conditions linked to elevated serum vitamin B12 blood levels.

As part of these additional tests of the results for children, we performed a linear regression analysis between serum vitamin B12 values and the different factors that might have contributed to selection of some subgroup of the population that for any reason had a much higher serum vitamin B12 level than the rest of the population. The strongest links between elevated serum vitamin B12 values in 2- to 20-year-old patients were found for the following factors: 1) patients that came from the hospital with ICD-10 diagnosis codes J00–J99 (diseases of the respiratory system) and 2) patients testing serum vitamin B12 on their own initiative. Patients with ICD-10 diagnosis codes J00–J99 directed from the outpatient treatment system were also found to be correlated with higher serum vitamin B12 blood level. In our opinion this can be at least partially explained by the fact that these patients presumably took vitamin pills and that many multivitamin formulations contain serum vitamin B12 with the range of 20–100% of the recommended daily dose (RDD). Patients who tested serum vitamin B12 on their own initiative could belong to the population subgroup that was either very health conscious or suspected that they needed to check serum vitamin B12 level due to some health problems like increased blood pressure, nausea, and headaches (Bahbouhi *et al*., 2023) or diet. In all of the above cases it was likely that such patients used serum vitamin B12 intake and thus might have higher serum vitamin B12 levels than a "serum vitamin B12 normal" individual.

In Figure 4 we analysed quantitative differences between the patients belonging to these subgroups and the rest of the patients. As can be seen in the figures, the median serum vitamin B12 level for these subgroups was indeed higher than in the rest of the population. However, the difference was only around 10% and thus was much smaller than the 50% increase of serum vitamin B12 level found in 2–10-year-old children.

DISCUSSION

The number of patients per age group varied from 173 in the age group below one year to 5589 in the age group 33–35 years old. The difference in the number of patients across age groups reflects the number of patients that performed B12 tests in the E. Gulbis Laboratory. In the Latvian population, there were fewer people in younger age groups due to the negative growth rate of the population since 1990 and fewer persons in the very old age groups of over 70 due

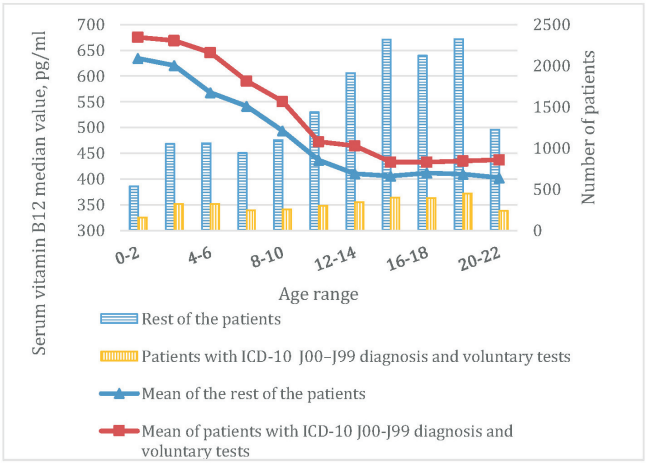

Fig. 4. Comparison of serum vitamin B12 median value for patients from a hospital with ICD-10 diagnosis codes J00–J99 (Diseases of the respiratory system) and patients testing B12 on their own initiative (line with square) with the rest of patients (line with triangles). The columns represent the number of patients in each of the groups.

to increased mortality, and with this correction taken in account, the number of patients per age group indicates that the serum vitamin B12 tests were most popular in the age group 25–50 years.

The relationship between age and serum vitamin B12 level (Fig. 3) shows three trends: distinct and strong serum vitamin B12 level peak for 3–9-year olds; somewhat lower serum vitamin B12 value in the age group from 13 to 27 years and almost stable serum vitamin B12 median value for those aged 30+. This observation corresponds to the elevated serum vitamin B12 values for children reported by the Disease Control & Center for Environmental Health (n.d.). The serum vitamin B12 blood level starts to increase after the age of 1 year, reaches its peak at 4–6 years, and slowly decreases to "adult level" at the age of 12. Median serum vitamin B12 level for young (18–39 years old) and old adults (60+ old) stays almost unchanged at around 460 pg/ml while the calculated RI for both the low-end value at 0,05 and the high-end value at 0,95 (of the Gaussian curve) starts to gradually diverge at the age of 55. This divergence with most probably can be attributed to the decline of the relative number of "serum vitamin B12 healthy" individuals at older age. Based on the results we do not consider that it would be appropriate to use the indirectly measured age specific RI for patients over 60 years.

The observed peak of serum vitamin B12 blood values for children is so pronounced that in our opinion it is important in the clinical interpretations of serum vitamin B12 test results. The increase of almost 50% as compared to older children (13–18 years) and young adults (18–39 years) is considerably higher than the experimental uncertainty and can influence decisions about serum vitamin B12 deficiency or diagnosis of high levels of serum vitamin B12 for children in this age group.

All the data we have analysed indicate that children in the age group of 2–10-years old indeed have elevated serum vi-

tamin B12 values as compared to the rest of the population. This is supported by data from USA (Disease Control & Center for Environmental Health, n.d.) where elevated data was reported for all ethnic groups studied — Hispanic, non-Hispanic white, and non-black. It is interesting to note that the prevalence of low serum vitamin B12 concentration (< 200 pg/ml) in the U.S. population was not reported only for children 1–11 years old due to a large relative statistical error just for this age group and no further conclusions about RI for children were reported. Elevated serum vitamin B12 values for children 1–11 years old were also reported in Bohn (2019) in Canada and in Abildgaard *et al*. (2022) in Denmark where in Abildgaard *et al*. (2022) elevated serum vitamin B12 RIs were suggested for children aged 0–11 years. In some other countries — parts of India (Singh *et al*., 2017), Nepal (Ng'Eno *et al*., 2017) and some parts of Brazil (Cobayashi *et al*., 2015), no elevation of serum vitamin B12 level for children was reported. When comparing results from different laboratories it should be noted that experimental uncertainties in serum vitamin B12 tests within 20% are considered to be normal and that results between different laboratories can differ even more.

According to population genetic studies (Krumiņa *et al*., 2018), the population of Latvia is located in the North European genetic cluster with the highest similarity to Estonians and is a mixture of genetic traits typical for Western Europe and Eastern Eurasia with only small differences between Latvian subpopulations. In the genome of Baltic natives, a greater similarity is found within the Western hunter-gatherer genome than in other modern populations (Mittnik *et al*., 2018). In line with population genetics data, our indirectly defined B12 RI for 2–10-year-old children should be in agreement with the European population, including the Danish population or mixed European population in Canada. Elevated serum vitamin B12 RI values for children 1–11-years old in Canada (Bohn, 2019) and for children aged 0–11 years in Denmark (Abildgaard *et al*., 2022) are in agreement.

Hypothetically, the decrease in total vitamin B12 in young people could be linked to excessive alcohol consumption. Alcohol has a negative effect on the liver, and as liver cells break down, not only liver-specific markers of liver damage, such as transaminases (ALT and GGT), but also, total B12 from reserves, enter the bloodstream. Reductions in RI in older adults may also be associated with chronic alcohol abuse. Without extra B12 intake, vitamin reserves are sufficient for some years. Persistent alcohol use inhibits the normal absorption of B12 due to damaged gastrointestinal mucosa, which may lead to a decrease in RI in the chronic alcohol consumer's group. Alcohol abuse is a problem in the Latvian population (McKee *et al*. 2000; Alcohol consumption estimated ..., 2023).

A healthy lifestyle could also be a reason for RIs elevation in young adults, as dietary supplements often contain higher B12 concentration than daily recommended intake (MacFarlane *et al*., 2014).

Considering the nature of the *in silico* analysis used in our work, we should consider the possibility that work the serum vitamin B12 values of children represent a subgroup of the children population with significantly elevated serum vitamin B12 values. The analysis of the patient data we performed has ruled out the possibility that this might be the case. This conclusion is also supported by findings in Gavars *et al*. (2022) that from all the serum vitamin B12 tests in Latvia, the number of the patients with lowered serum vitamin B12 level considerably exceeded the number of the patients with elevated serum vitamin B12 values. Serum vitamin B12 is increasingly being tested in connection with anaemia, which is again associated with low serum vitamin B12 levels (Abu *et al*., 2020; Omer *et al*., 2022). We have not identified any medical disorder in young children that can be associated with considerably elevated serum vitamin B12 levels and that is sufficiently widespread to be able to influence the results. An increased serum vitamin B12 level might be due to the intake of serum vitamin B12 vitamin supplements — either in pure form or as part of a multivitamin formulation (Andrès *et al*., 2013; Channer-Wallen *et al*., 2022). This can explain the elevated serum vitamin B12 level for children, if we assume that most of the children below 8 years took Vitamin B12 either regularly or prior to tests and that after 10 years of age this behaviour ceased. Since this is very unlikely, we conclude that with a very high level of certainty the elevated B12 level is indeed characteristic for children aged 2–10 years.

Based on our research we recommend using a different serum vitamin B12 RI for children aged from 2 to 10 years in the interpretation of serum vitamin B12 tests for health care purposes. There are indications that the elevation of serum vitamin B12 level for children might be country specific and thus local RI are to be preferred when possible.

CONCLUSIONS

From a large number of Latvia patient data, we calculated median serum vitamin B12 values and serum vitamin B12 RIs for different age groups with a temporal resolution of two years. Children aged 2–10 years showed considerably higher serum vitamin B12 levels than the rest of the population and we recommend using different higher RIs in interpreting serum vitamin B12 clinical tests for children. For the rest of the population, serum vitamin B12 blood level remains almost constant for all other age groups with small minima at 16 years of age.

ETHICS

The study used anonymised data of serum vitamin B12 blood level measurements accumulated in E. Gulbis laboratory and was approved by the Ethics Board of Rīga Stradiņš University, the approval decision 13.05.2022 number 2-PĒK-4/288/2022. This work was carried out in accordance with the Code of Ethics of the World Medical Association.

REFERENCES

Abildgaard, A., Knudsen, C. S., Hoejskov, C. S., Greibe, E., Parkner, T. (2022). Reference intervals for plasma vitamin B12 and plasma/serum methylmalonic acid in Danish children, adults and elderly. *Clin. Chim. Acta*, **525**, 62–68. DOI: 10.1016/j.cca.2021.12.015.

Abu, B. A. Z., Raubenheimer, J. E., Louise van den Berg, V. (2020). Iron-focussed nutritional status of mothers with children (6–59 months) in rural northern Ghana. *Heliyon*, **6** (6). DOI: 10.1016/j.heliyon.2020.e04017.

Alcohol consumption estimated to cause millions in losses to state. https://eng.lsm.lv/article/economy/economy/22.11.2023-alcohol-consumption-estimated-to-cause-millions-in-losses-to-state.a532577/ (accessed 04.04.2024).

Allen, L. H. (2012). Vitamin B-12. *Adv. Nutr.*, **3** (1), 54–55. DOI:10.3945/an.111.001370.

Al-Musharaf, S., McTernan, P. G., Hussain, S. D., Aleisa, K. A., Alnaami, A. M., Wani, K., Saravanan, P., Al-Daghri, N. (2021). Prevalence and indicators of vitamin B12 insufficiency among young women of childbearing age. *Int. J. Environ. Res. Publ. Health*, **18** (1), 1–13. DOI: 10.3390/ijerph18010001.

Andrès, E., Serraj, K., Zhu, J., Vermorken, A. J. M. (2013). The pathophysiology of elevated vitamin B12 in clinical practice. *QJM*, **106** (6), 505–515). DOI: 10.1093/qjmed/hct051.

Arzideh, F., Wosniok, W., Gurr, E., Hinsch, W., Schumann, G., Weinstock, N., Haeckel, R. (2007). A plea for intra-laboratory reference limits. Part 2. A bimodal retrospective concept for determining reference limits from intra-laboratory databases demonstrated by catalytic activity concentrations of enzymes. *Clin. Chem. Labor. Med.,* **45** (8), 1043–1057. DOI: 10.1515/CCLM.2007.25

Bahbouhi, I., Aboudourib, M., Hocar, O., Amal, S. (2023). Vitamin B12 induced acneiform eruption. *Heliyon*, **9** (5), e16120. DOI: 10.1016/j.heliyon.2023.e16120.

Bohn, M. K., Higgins, V., Asgari, S., Leung, F., Hoffman, B., Macri, J., Adeli, K. (2019). Paediatric reference intervals for 17 Roche cobas 8000 e602 immunoassays in the CALIPER cohort of healthy children and adolescents. *Clin. Chem. Labor. Med.,* **57** (12), 1968–1979.

Box, G. E. P., Cox, D. R. (1964). An Analysis of Transformations. *J. Roy. Soc.,* Series *B* (Methodological), **26** (2), 211–252.

Broz, P., Rajdl, D., Racek, J., Trefil, L., Stehlik, P. (2022). Effect of beer consumption on methylation and redox metabolism. *Physiol. Res.,* **71** (4), 573–582. DOI: 10.33549/physiolres.934863.

Channer-Wallen, T., Dawson, P., Thomas-Brown, P. G., Gossell-Williams, M. (2022). Lack of association between serum vitamin B12 and nocturnal sleep parameters following cyanocobalamin supplementation in healthy adults. *Heliyon*, **8** (2), e08831. DOI: 10.1016/j.heliyon.2022.e08831.

Cobayashi, F., Tomita, L. Y., Augusto, R. A., D'Almeida, V., Cardoso, M. A. (2015). Genetic and environmental factors associated with vitamin B12 status in Amazonian children. *Publ. Health Nutr.,* **18** (12), 2202–2210. DOI: 10.1017/S1368980014003061.

Devi, A., Rush, E., Harper, M., Venn, B. (2018). Vitamin B12 status of various ethnic groups living in New Zealand: An analysis of the adult nutrition survey 2008/2009. *Nutrients*, **10** (2). DOI: 10.3390/nu10020181.

Disease Control, C., Center for Environmental Health, N. ((2012). *Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population*. 495 pp. https://www.cdc.gov/nutritionreport/pdf/nutrition_book_complete508_final.pdf (accessed 10.04.2024).

Esnafoglu, E., Ozturan, D. D. (2020). The relationship of severity of depression with homocysteine, folate, vitamin B12, and vitamin D levels in children and adolescents. *Child Adolesc. Mental Health*, **25** (4), 249–255. DOI: 10.1111/camh.12387.

Fatima, M. T., Bhat, A. A., Nisar, S., Fakhro, K. A., Al-Shabeeb Akil, A. S. (2023). The role of dietary antioxidants in type 2 diabetes and neurodegenerative disorders: An assessment of the benefit profile. *Heliyon*, **9** (1), e12698. DOI: 10.1016/j.heliyon.2022.e12698.

Flores-Guerrero, J. L., Minoviã, I., Groothof, D., Gruppen, E. G., Riphagen, I. J., Kootstra-Ros, J., Muller Kobold, A., Hak, E., Navis, G., Gansevoort, R. T., de Borst, M. H., Dullaart, R. P. F., Bakker, S. J. L. (2020). Association of plasma concentration of vitamin B12 with all-cause mortality in the general population in the Netherlands. *JAMA Network Open*, **3** (1), e1919274. DOI: 10.1001/jamanetworkopen.2019.19274.

Fuzo, C. A., da Veiga Ued, F., Moco, S., Cominetti, O., Métairon, S., Pruvost, S., Charpagne, A., Carayol, J., Torrieri, R., Silva, W. A., Descombes, P., Kaput, J., Monteiro, J. P. (2021). Contribution of genetic ancestry and polygenic risk score in meeting vitamin B12 needs in healthy Brazilian children and adolescents. *Sci. Rep.,* **11** (1), 11992. DOI: 10.1038/s41598-021-91530-7.

Gavars, D., Perminov, D., Tauckels, E., Lindenberga, I., Tutāne, A., Auce, A. (2022). Extraction of B12 reference intervals from a large amount of general patient data. *Proc. Latv. Acad. Sci., Section B,* **76** (3), 333–337. https://www.sciendo.com/article/10.2478/prolas-2022-0050. DOI: 10.2478/prolas-2022-0050.

Gilsing, A. M. J., Crowe, F. L., Lloyd-Wright, Z., Sanders, T. A. B., Appleby, P. N., Allen, N. E., Key, T. J. (2010). Serum concentrations of vitamin B12 and folate in British male omnivores, vegetarians and vegans: Results from a cross-sectional analysis of the EPIC-Oxford cohort study. *Eur. J. Clin. Nutr.,* **64** (9), 933–939. DOI: 10.1038/ejcn.2010.142.

González-Gross, M., Benser, J., Breidenassel, C., Albers, U., Huybrechts, I., Valtueña, J., Spinneker, A., Segoviano, M., Widhalm, K., Molnar, D., Moreno, L. A., Stehle, P., Pietrzik, K. (2012). Gender and age influence blood folate, vitamin B12, vitamin B6, and homocysteine levels in European adolescents: The Helena Study. *Nutr. Res.,* **32** (11), 817–826. DOI: 10.1016/j.nutres.2012.09.016.

Hao, L., Ma, J., Zhu J., Stampfer, M. J., Tian, Y., Willett, W. C., Li, Z. (2007). Vitamin B-12 deficiency is prevalent in 35-to 64-Year-Old Chinese adults 1,2. *J. Nutr.,* **137** (5), 1278–1285. https://academic.oup.com/jn/article/137/5/1278/4664612.

Henry, O. R., Benghuzzi, H., Taylor, H. A., Tucci, M., Butler, K., Jones, L. (2012). Suppression of homocysteine levels by vitamin B12 and folates: Age and gender dependency in the Jackson heart study. *Amer. J. Med. Sci.,* **344** (2), 110–115. DOI: 10.1097/MAJ.0b013e31823782a5.

Horowitz G. L., Altaie S., Boyd J. C., *et al*. (2010). EP28-A3c: Defining, establishing, and verifying reference intervals in the clinical laboratory; Approved Guideline, Third Edition. Clinical and Laboratory Standards Institute, 28 October, 12. www.clsi.org. DOI: 56238-682-4.

Iedzīvotāji pēc tautības gada sākumā – Tautība un Laika periods. (n.d.). https://data.stat.gov.lv/pxweb/lv/OSP_PUB/START__POP__IR__IRE/IRE010/table/tableViewLayout1/ (accessed 11.04.2023).

Jiang, W., Men, S., wen, X., Yuan, X., Pu, D., Liu, X., Jia, X., Wang, C. (2020). A preliminary study for the establishment of a reference interval for vitamin B12 in China after performance verification of a second-generation ECLIA kit. *J. Clin. Labor. Anal.,* **34** (5), e23165. https://pubmed.ncbi.nlm.nih.gov/31907976/. DOI: 10.1002/jcla.23165.

Kim, Y. N., Hwang, J. H., Cho, Y. O. (2018). One-half of Korean adults studied had marginal vitamin B 12 status assessed by plasma vitamin B 12. *Nutr. Res.,* **50**, 37–43. DOI: 10.1016/j.nutres.2017.10.004.

Krūmiņa, A., Pliss, L., Zariņa, G., Puzuka, A., Zariņa, A., Lāce, B., Elferts, D., Khrunin, A., Limborska, S., Kloviņš, J., Gailīte-Piekuse, L. (2018). Population genetics of Latvians in the context of admixture between North-Eastern European ethnic groups. *Proc. Latvian Acad. Sci., Section B,* **72** (3), 131–151. DOI: 10.2478/prolas-2018-0025

Laufer, E. M., Hartman, T. J., Baer, D. J., Gunter, E. W., Dorgan, J. F., Campbell, W. S., Clevidence, B. A., Brown, E. D., Albanes, D., Judd, J. T., Taylor, P. R. (2004). Effects of moderate alcohol consumption on folate and vitamin B12 status in postmenopausal women. *Eur. J. Clin. Nutr.,* 58 (11), 1518–1524. DOI: 10.1038/sj.ejcn.1602002.

Mathias, M. G., Coelho-Landell, C. de A., Scott-Boyer, M. P., Lacroix, S., Morine, M. J., Salomão, R. G., Toffano, R. B. D., Almada, M. O. R. do V., Camarneiro, J. M., Hillesheim, E., *et al.* (2018). Clinical and vitamin re-

sponse to a short-term multi-micronutrient intervention in Brazilian children and teens: From population data to interindividual responses. *Mol. Nutr. Food Res.,* **62** (6), e1700613. DOI: 10.1002/mnfr.201700613.

MacFarlane, A. J., Shi, Y., Greene-Finestone, L. S. (2014). High-dose compared with low-dose vitamin B-12 supplement use is not associated with higher vitamin B-12 status in children, adolescents, and older adults. *J. Nutr.,* **144** (6), 915–920. DOI: 10.3945/jn.113.190256.

McKee, M., Pomerleau, J., Robertson, A., Pudule, I., Grinberga, D., Kadziauskiene, K., Abaravicius, A., Vaask, S. (2000). Alcohol consumption in the Baltic Republics. *J. Epidemiol. Commun. Health*, **54** (5), 361–366. DOI: 10.1136/jech.54.5.361.

Miki, A., Kinno, R., Ochiai, H., Kubota, S., Mori, Y., Futamura, A., Sugimoto, A., Kuroda, T., Kasai, H., Yano, S., Hieda, S., Kokaze, A., Ono, K. (2021). Sex differences in the relationship of serum vitamin B1 and B12 to dementia among memory clinic outpatients in Japan. *Front. Aging Neurosci.,* **13,** 667215. DOI: 10.3389/fnagi.2021.667215.

Mittnik, A., Wang, C. C., Pfrengle, S., Daubaras, M., Zariņa, G., Hallgren, F., Allmäe, R., Khartanovich, V., Moiseyev, V., Tõrv, M., *et al.* (2018). The genetic prehistory of the Baltic Sea region. *Nat. Commun.,* **9** (1), 442. DOI: 10.1038/s41467-018-02825-9.

Ng'Eno, B. N., Perrine, C. G., Whiteheadjr, R. D., Subedi, G. R., Mebrahtu, S., Dahal, P., Jefferds, M. E. D. (2017). High prevalence of vitamin B12 deficiency and no folate deficiency in young children in Nepal. *Nutrients*, **9** (1), 72. DOI: 10.3390/nu9010072.

O'Logbon, J., Crook, M., Steed, D., Harrington, D. J., Sobczyñska-Malefora, A. (2021). Ethnicity influences total serum Vitamin B12 concentration: A study of Black, Asian and White patients in a primary care setting. *J. Clin. Pathol.*, **75** (9), 598–604. https://pubmed.ncbi.nlm.nih.gov/33952588/. DOI: 10.1136/jclinpath-2021-207519.

Omer, A., Hailu, D., Nigusse, G., Mulugeta, A. (2022). Magnitude and morphological types of anemia differ by age among under five children: A facility-based study. *Heliyon*, **8** (9), e10494. DOI: 10.1016/j.heliyon.2022.e10494.

Orton, D. J., Naugler, C., Sadrzadeh, S. M. H. (2016). Fasting time and vitamin B12 levels in a community-based population. *Clin. Chim. Acta*, **458**, 129–132. DOI: 10.1016/j.cca.2016.05.005.

Ozarda, Y., Ichihara, K., Jones, G., Streichert, T., Ahmadian, R. (2021). Comparison of reference intervals derived by direct and indirect methods based on compatible datasets obtained in Turkey. *Clin. Chim. Acta*, **520**, 186–195. DOI: 10.1016/j.cca.2021.05.030.

Parackal, S., Smith, C., Skidmore, P. (2020). Diet quality, nutrient intakes and biochemical status of New Zealand women of childbearing age according to alcohol consumption patterns. *Publ. Health Nutr.,* **23** (16), 2952–2962. DOI: 10.1017/S1368980019003781.

Referenzinstitut für Bioanalytik. (2020). Vitamin B12 external quality control dataset April 2020. https://www.rfb.bio/ (accessed 04.04.2024).

Risch, M., Meier, D. W., Sakem, B., Medina Escobar, P., Risch, C., Nydegger, U., Risch, L. (2015). Vitamin B12 and folate levels in healthy Swiss senior citizens: A prospective study evaluating reference intervals and decision limits. *BMC Geriatrics*, **15** (1), DOI: 10.1186/s12877-015-0060-x.

Roche Diagnostics. (2010). Manual: Vitamin B12 analysis using Elecsys. https://diagnostics.roche.com/global/en/products/lab/elecsys-vitamin-b12-ii-cps-000536.html (accessed 04.04.2024).

Silva, L. L. S., Fawzi, W. W., Cardoso, M. A. (2019). Serum folate and Vitamin B 12 status in young Brazilian children. *Publ. Health Nutr.,* **22** (7), 1223–1231. DOI: 10.1017/S1368980019000193.

Singh, S., Geddam, J. J. B., Reddy, G. B., Pallepogula, D. R., Pant, H. B., Neogi, S. B., John, N., Kolli, S. R., Doyle, P., Kinra, S., Ness, A., Murthy, G. V. S. (2017). Folate, vitamin B12, ferritin and haemoglobin levels among women of childbearing age from a rural district in South India. *BMC Nutr.*, **3** (1), 50. DOI: 10.1186/s40795-017-0173-z.

Soh, Y., Won, C. W. (2020). Association between frailty and vitamin B12 in the older Korean population. *Medicine*, **99** (43), e22327. DOI: 10.1097/MD.0000000000022327.

Wosniok, W., Haeckel, R. (2019). A new indirect estimation of reference intervals: Truncated minimum chi-square (TMC) approach. *Clin. Chem. Labor. Med.,* **57** (12), 1933–1947, DOI: 10.1515/cclm-2018-1341.

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B12 VITAMĪNA SERUMA LĪMEŅA ASINĪS ATKARĪBA NO VECUMA LATVIJAS POPULĀCIJĀ

Ar *in silico* metodes palīdzību tika noteikti B12 vitamīna līmeņa serumā references intervāli un mediānās vērtības Latvijas iedzīvotājiem dažādās vecuma grupās ar divu gadu soli. Pētījumā tika izmantoti anonimizēti E. Gulbja laboratorijā uzkrātie liela apjoma B12 analīžu dati. Dati reprezentē Latvijas iedzīvotāju kopumu. Tika izmantota E. Gulbja laboratorijā izstrādāta netiešā *in silico* metode B12 vitamīna līmeņa serumā asinīs references intervālu noteikšanai. Konstatēta spēcīga B12 vitamīna līmeņa paaugstināšanās seruma asinīs 2–10 gadus veciem bērniem. Secinājumos ieteikts noteikt atšķirīgu seruma B12 vitamīna references intervālu bērniem.