Establishing reference intervals for serum ferritin and vitamin B_{12} using a modified Hoffmann's approach

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Introduction

Age and/or sex specific population-based reference intervals (RIs) are rarely available or difficult to establish in Clinical Laboratories. With an increased focus on the between-method standardization and harmonization of test results, the development of universal RIs for standardized and harmonized assays may help laboratories to improve patient care. Both serum ferritin and serum vitamin B_{12} (B_{12}) concentrations vary with age and gender, yet unified RIs are often applied. Both lower and upper limits for these markers are clinically important, since low values suggest deficiency leading to anaemia, and high values may reflect iron overloading/acute phase (ferritin) or abnormalities in vitamin B_{12} binding proteins e.g. as seen with some cancers (B_{12}). Therefore, accurate and subgroup-specific RIs should be applied.

Aim

To establish RIs for ferritin and B_{12} using a modified Hoffmann's approach.[1]

A modified Hoffmann's method

In 1963, Hoffmann described a simple, indirect method of calculating RIs using existing patient data from a laboratory database, named 'probability paper method'.[2] Later, the first computerized software based on Hoffmann's approach was developed.[3] In brief, Chauvenet's criteria were used for the detection of outliers (Figure 1). Following the outliers removal, the cumulative frequency of each test result was determined. Values from the linear portion of the cumulative frequency graph were used for computing the best fitting linear regression equation, $y_i=\alpha*\chi+\beta+\varepsilon_i$ (Figure 3). The RIs were then determined from the linear regression equation following extrapolation of the preceding curve. RI was calculated (for x=2.5% and 97.5%): **$RI_{min}= \alpha*2.5+\beta$, $RI_{max}= \alpha*97.5+\beta$.** When the source data distribution is significantly skewed, a Box-Cox transformation may be applied (Figure 2), with back transformation after the linear portion is calculated from the transformed data. In this work a fully computerised and validated method, with new functions and algorithms added was used.

Methods

All ferritin results processed between Aug 2014-Jul 2015 and B_{12} processed between Jan-June 2013 on *i*2000$_{SR}$ (Abbott Diagnostics) from a population served by Guy's and St. Thomas' Hospitals in London, UK were used to calculate RIs. Data was partitioned in accordance with literature based knowledge about sex/age related differences in these markers.

Results

The RIs with percentage of values below and above the cut-offs are shown in Table 1. Because of low sample numbers, separate RIs for age group 0-12 months were not calculated.

Size of data:	5514
Number of outliers:	258
Maximum Error Threshold:	0.465
Maximum Error:	0.464
% of data in linear range:	90.487
Start cut point:	2.702
End cut point:	93.189
RI:	[9.059, 13.91]
Regression:	y = (0.051)x + (8.932)
Boxcox:	c=0, λ=0.2
inversed RI:	[175.789, 773.72]
CI:	[153.958, 200.028], [701.429, 851.851]
% of data in calculated RI:	83.079
% of data above the upper limit of calculated RI:	12.786
% of data below the lower limit of calculated RI:	4.135
Mean of all data:	521.011
Median of all data:	390
SD of all data:	482.695
Mean (linear region):	399.597
Median (linear region):	370
SD (linear region):	156.253

Table 1. An example of a representative, selected report (total B_{12}, age group 60+ yrs)

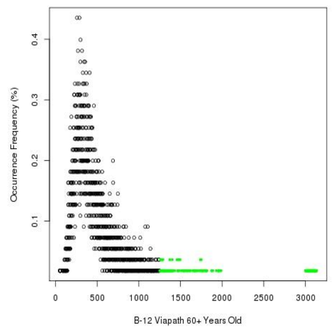

Figure 1. An example of a dot-plot showing 'good' data (black dots) and outliers (green dots). Data for total B_{12}, age group 60+ yrs.

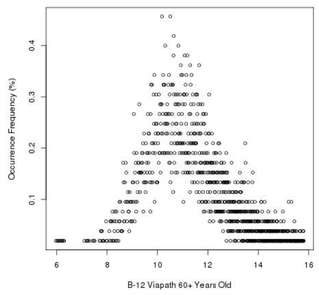

Figure 2. An example of a dot-plot (no outliers) after Box-Cox transformation (total B_{12}, age group 60+ yrs)

Ferritin					Total B_{12}				
Partition group; gender/age	Data size	RIs (ng/mL)	% below lower limit	% above upper limit	Partition group by age	Data size	RIs (ng/L)	% below lower limit	% above upper limit
M/1-5 yrs	845	**9 - 70**	5.2	30.0	0-19 yrs	720	**224 - 1001**	5.7	10.8
F/1-5 yrs	488	**10 - 73**	3.3	32.2	6-19 yrs	624	**218 - 878**	5.6	10.6
M/6-11 yrs	899	**14 - 85**	3.1	28.5	20-59 yrs	11641	**194 - 829**	4.8	10.1
F/6-11 yrs	802	**13 - 74**	3.5	38.6	60+ yrs	5514	**176 - 774**	4.1	12.8
M/12-19 yrs	1122	**17 - 143**	3.5	31.7					
F/12-19 yrs	1760	**7 - 75**	3.7	22.9					
M/20-55 yrs	9767	**34 - 314**	6.8	15.4					
F/20-55 yrs	25823	**9 - 102**	5.7	13.9					
M/56+ yrs	9360	**25 - 503**	7.6	12.0					
F/56+ yrs	11575	**19 - 262**	6.8	15.3					

Table 2. Age and/or sex related RIs for serum ferritin and total B_{12}

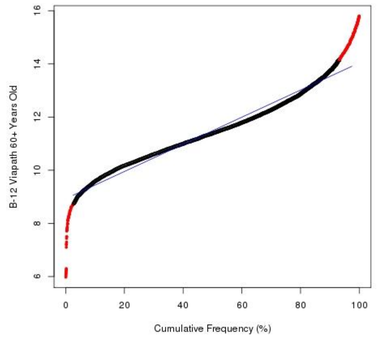

Figure 3. An example of cumulative frequencies (dots) and regression line (total B_{12}, age group 60+ yrs)

Discussion: The RIs for serum ferritin and B_{12}, calculated using a modified Hoffmann's approach are consistent with RIs established using harmonized methods and may serve as universal RIs for other laboratories using the same methodology. They incorporate variations related to age, gender, method and the population being tested. The variations in upper limits for ferritin are of particular interest in view of iron overloading and deserve further investigations. Application of these RIs can assist with a better assessment of iron and vitamin B_{12} status.

References:

1. Katayev A, Fleming JK, Luo D, Fisher AH, Sharp TM. Reference intervals data mining: no longer a probability paper method. *Am.J.Clin.Pathol.* 2015;**143**:134-42.

2. Hoffmann RG. Statistics in the practice of medicine. *JAMA* 1963;**185**:864-73.

3. Katayev A, Balciza C, Seccombe DW. Establishing reference intervals for clinical laboratory test results: is there a better way? *Am.J.Clin.Pathol.* 2010;**133**:180-6.