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Laboratory Data: Issues with Multiple Reference Ranges and Methods of Normalization

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ABSTRACT

Laboratory data is required in almost every clinical trial for safety reasons, especially in multi-center trials, where different laboratories are involved. This can lead to widely different laboratory results and thus to different reference ranges. Therefore, combining data from different laboratories can be a huge challenge. In order to make results from different laboratories comparable, they need to be normalized. A commonly used method for normalizing laboratory values transforms the data and normalizes it to a common reference range. This transformation preserves the distance of the laboratory value from the lower limit of normal as a multiple of the specified normal range. This presentation will introduce this method, provide examples of the selection of the common reference range and show differences in the resulting outputs. Furthermore, it will demonstrate its limitations and discuss alternative methods.

INTRODUCTION

Laboratory data is routinely collected in clinical trials and provides important information regarding the safety precautions for patients. They are objective as well as related to organ functionality. Occurring abnormal findings can be indicators for systemic toxicities and can provide an early indication of undesirable clinical signs. Especially in phase III studies, multi-center clinical trials are very common. The required sample size is usually very large and difficult for one center alone to handle. Besides the many advantages multi-center clinical trials offer, they bring about new challenges to deal with. Mainly, operational questions such as protocol compliance and comparable patient care arise. Furthermore, the analysis of such trials is a big challenge for data scientists.

Although central laboratories are often used to process lab specimens, there are still studies where additional laboratories are used. Laboratories involved in the trials use different manufacturing equipment and assay procedures, which therefore result in diverse results. Moreover, laboratories could replace their equipment or alter their procedures during a clinical trial. This could lead to very divergent laboratory results and different reference ranges. A reference range is a range of values that includes upper and lower limits which are estimated to enclose a specified percentage (usually the central 95%) in laboratory tests based on a group of healthy people. It serves as a basis to compare and interpret a set of test results for a particular patient. Pooling laboratory data from different laboratories to obtain summary statistics can be challenging. The heterogenous units and reference ranges of measurements used by different laboratories are some of those challenges. The major issue is that the clinical interpretation is critical if laboratory values with different normal reference ranges and/or units were analyzed together. Normalization means that values taken from different laboratories are transformed to make them directly comparable, but to keep existing differences that can occur between different groups, for example between female and male subjects.

In this paper, we will be discussing the location-scale method first introduced by Chuang-Stein 1992 on how to normalize laboratory data. We will provide various examples of the common reference range and show how the resulting outputs differ depending on the choice of a common reference range. Furthermore, we will discuss the limitations of this method and present alternative methods that could be used to handle the varying laboratory data.

THE LOCATION-SCALE MODEL BY CHUANG-STEIN

A method for normalizing laboratory data based on reference ranges was first introduced by Chuang-Stein. The idea is to normalize all values in relation to a selected set of reference ranges. Chuang-Stein's proposal is to normalize an assay value relative to a chosen standard reference range, which can be expressed by the following formula:

$$value_{std} = (value - LLN) * \frac{ULN_{std} - LLN_{std}}{ULN - LLN} + LLN_{std}$$

where lower limit of normal (LLN) and upper limit of normal (ULN) are the local limits and LLN_{std} and ULN_{std} are the limits of the common reference range used and which should be the standard range for the assay. Value represents an assay value. This transformation preserves the distance of the laboratory value from the lower limit of normal as a multiple of the specified normal range, i.e.

$$\frac{value - LLN}{ULN - LLN} = \frac{value_{std} - LLN_{std}}{ULN_{std} - LLN_{std}}$$

This normalization model is particularly suitable for values that are normally distributed. One disadvantage is that normalized values can become negative. In such cases this normalization function is not appropriate.

ALTERNATIVE METHODS OF DATA NORMALIZATION

SCALE MODEL

This normalization model can either be based on upper reference limits or lower reference limits. It is more conventional to use upper reference limits. One reason is that the conventional application in this model is usually used in clinical chemistry and therefore, the upper reference limits have more clinical relevance. The scale model is formed in the following scale normalization formula:

$$value_{std} = value * \frac{ULN_{std}}{ULN}$$

where ULN, ULN_{std} and values are defined like in the location-scale model. Publications recommend using the scale normalization model particularly in clinical chemistry. It is suitable for skewed distributions of assay values. The main disadvantage of this method is that it focuses mainly on the high ends of the laboratory values and is therefore not treating the lower limits and the upper limits in an equivalent manner. Detailed information on this method can be found in the publication of Karvanen et al. 2003.

GENIE SCORE METHOD

The Genies Score method was proposed by Sogliero-Gilbert et al. in 1986. This Score method provides the severity of abnormality in each body function. It is a summary statistic procedure (for a functional group of parameters) from a weighted linear combination of absolute normalized deviations in the normal range in an individual patient. If an observation is outside the normal reference range, the Genie Score method results in a value that differs from zero. For all observations that fall within the normal reference range, the score is zero. All Genie scores are greater than or equal to zero. Therefore, higher Genie Scores reflect an increased severity of abnormalities. As a result, you can get an analysis of the severity of laboratory abnormality for all the different body functions or a combined overall abnormality index. A disadvantage is that Genie Scores only have significance in a comparative sense and are only used for decision making purposes. Therefore, their use for generating summary statistics to be implemented in the various laboratories, is limited.

CHOOSING THE COMMON REFERANCE RANGE

Choosing the common reference range has an important impact on the results and the quality of data normalization. There is a lack of consensus on how common reference ranges should be mathematically constructed. This leads to diverse ranges, even in the same set of laboratory results. Chuang-Stein implemented the location-scale model in 1992 and used the reference range from the center with the highest patient enrollment as the common reference range. This approach may lead to standardized values smaller than zero. Ruvuna et al. suggested using the percentile method to create the common reference range. The general conceptual method involves finding a sample percentile by using the limit of the population percentage excluding a specific value. Another common application is to use the maximum lower limit of normal (max (LLN)) and the minimum upper limit of normal (min (ULN)). This approach avoids standardized values smaller than zero but does not work if the reference ranges are disjunct. Moreover, it leads to narrow ranges and reduces the absolute change. Another possibility is to use the reference range with the maximum upper limit of normal as the common one. If more than one maximum of upper limit of normal exists, then the reference range with the maximum lower limit of normal within these reference ranges is used.

ANALYSIS

In the following chapter an analysis using various selections for the common reference range is described. The Chuang-Stein model described above is applied on generated data. Box plots are used to display the various results in a descriptive manner.

DATA

For this analysis generated laboratory data is used. The database for this paper comprises of 239 subjects, 198 females and 41 males. It contains information from three laboratory parameters: Aspartate Aminotransferase, Creatinine and Hemoglobin.

Aspartate Aminotransferase is an enzyme which is important for amino acid metabolism and is used to determine liver function. Creatinine is produced in the liver and has a higher value for patients with a higher muscle mass. It can be measured in the blood and can be used to calculate the estimated glomerular filtration rate (eGFR), which in turn measures the renal function. Hemoglobin is a protein in the red blood cells which transports oxygen from the lungs to other parts of the body. Men have higher reference ranges for hemoglobin than women.

For each subject the results of the parameters are stored in three subsequent visits: Visit 1, Visit 5 and Visit 7. Not every subject has observations for all of the three visits. Along with the results, information about the relative treatment day and the laboratory reference ranges with units is stored. In total there are 1810 observations available in the data set. See Table 1 below.

Table 1: First 21 observations of the laboratory analysis data

	Subject Identifier for the Study	Sex	Parameter	Parameter Code	Analysis Visit	Analysis Relative Day	Analysis Value	Reference Range
1	112	F	Creatinine (umol/L)	CREAT	VISIT 1	-1	42.2	35.36-61.88
2	112	F	Creatinine (umol/L)	CREAT	VISIT 5	22	55.04	35.36-61.88
3	112	F	Creatinine (umol/L)	CREAT	VISIT 7	43	51.04	35.36-61.88
4	112	F	Hemoglobin (g/dL)	HGB	VISIT 1	-8	13.1	12-16
5	112	F	Hemoglobin (g/dL)	HGB	VISIT 5	22	14.1	12-16
6	112	F	Hemoglobin (g/dL)	HGB	VISIT 7	43	11.5	12-16
7	125	F	Aspartate Aminotransferase (U/L)	AST	VISIT 1	-3	33	8-40
8	125	F	Aspartate Aminotransferase (U/L)	AST	VISIT 5	20	24	8-40
9	125	F	Aspartate Aminotransferase (U/L)	AST	VISIT 7	41	20	8-40
10	125	F	Creatinine (umol/L)	CREAT	VISIT 1	-3	77	70-106
11	125	F	Creatinine (umol/L)	CREAT	VISIT 5	20	72	70-106
12	125	F	Creatinine (umol/L)	CREAT	VISIT 7	41	70	70-106
13	127	M	Aspartate Aminotransferase (U/L)	AST	VISIT 1	-6	31	0-38
14	127	M	Aspartate Aminotransferase (U/L)	AST	VISIT 5	34	21	0-38
15	127	M	Aspartate Aminotransferase (U/L)	AST	VISIT 7	54	20	0-38
16	127	M	Creatinine (umol/L)	CREAT	VISIT 1	-6	59.88	61.88-106.08
17	127	M	Creatinine (umol/L)	CREAT	VISIT 5	34	59.88	61.88-106.08
18	127	M	Creatinine (umol/L)	CREAT	VISIT 7	54	72.72	61.88-106.08
19	127	M	Hemoglobin (g/dL)	HGB	VISIT 1	-6	12.3	14-18
20	127	M	Hemoglobin (g/dL)	HGB	VISIT 5	34	8.7	14-18
21	127	M	Hemoglobin (g/dL)	HGB	VISIT 7	54	8.2	14-18

Table 1 shows the first 21 observations of the laboratory analysis data set. All additional observations have the same structure. The data shows different reference ranges for the same parameters present. More information on the data, specifically the range of the different reference ranges, is shown in the tables below.

Table 2: Overview of the different reference ranges per parameter

	Parameter	Reference Range	Frequency Count
1	Aspartate Aminotransferase (U/L)	0-32	51
2	Aspartate Aminotransferase (U/L)	0-38	24
3	Aspartate Aminotransferase (U/L)	13-33	41
4	Aspartate Aminotransferase (U/L)	3-37	24
5	Aspartate Aminotransferase (U/L)	5-35	20
6	Aspartate Aminotransferase (U/L)	5-37	11
7	Aspartate Aminotransferase (U/L)	8-40	65
8	Creatinine (umol/L)	35.36-61.88	33
9	Creatinine (umol/L)	44-115	33
10	Creatinine (umol/L)	44.2-176.8	14
11	Creatinine (umol/L)	44.2-79.56	30
12	Creatinine (umol/L)	53.04-141.44	24
13	Creatinine (umol/L)	53.04-97.24	29
14	Creatinine (umol/L)	61.88-106.08	10
15	Creatinine (umol/L)	70-106	21
16	Hemoglobin (g/dL)	10-15	24
17	Hemoglobin (g/dL)	11-15	17
18	Hemoglobin (g/dL)	11-16	49
19	Hemoglobin (g/dL)	11.3-15.2	1
20	Hemoglobin (g/dL)	11.5-15	16
21	Hemoglobin (g/dL)	12-15	8
22	Hemoglobin (g/dL)	12-16	75
23	Hemoglobin (g/dL)	12-18	5
24	Hemoglobin (g/dL)	14-18	10

Table 2 shows the various laboratory ranges for each parameter. For all three parameters, a number of reference ranges occurs in the data set. For Aspartate Aminotransferase seven other reference ranges are present, for Creatinine there are eight different reference ranges and for Hemoglobin even nine reference ranges occur. In the column 'Frequency Count' it is displayed how often each reference range is present in the data. Most reference ranges are present for more than one subject. One exception is 11.3-15.2. This value is present only once in the data. The most frequent ranges for each laboratory parameter are important to choose the common reference ranges as a method for normalization.

Table 3: Overview of reference ranges of hemoglobin per gender

	Sex	Parameter	Reference Range	Frequency Count
1	F	Hemoglobin (g/dL)	10-15	19
2	F	Hemoglobin (g/dL)	11-15	17
3	F	Hemoglobin (g/dL)	11-16	40
4	F	Hemoglobin (g/dL)	11.3-15.2	1
5	F	Hemoglobin (g/dL)	11.5-15	16
6	F	Hemoglobin (g/dL)	12-15	8
7	F	Hemoglobin (g/dL)	12-16	71
8	M	Hemoglobin (g/dL)	10-15	5
9	M	Hemoglobin (g/dL)	11-16	9
10	M	Hemoglobin (g/dL)	12-16	4
11	M	Hemoglobin (g/dL)	12-18	5
12	M	Hemoglobin (g/dL)	14-18	10

Table 3 shows the laboratory ranges for hemoglobin according to gender. Men and women have dissimilar average hemoglobin levels and therefore, the reference ranges for hemoglobin are varied. This laboratory data set contains seven different reference ranges for hemoglobin for female subjects and five different reference ranges for male subjects. The reference ranges are higher in the male population. In the table above the frequency count for the reference ranges separated by sex is displayed. The most present range for female subjects is 12-16 and for male subjects 14-18.

Table 4: Summary statistics of the analysis data by parameter

The MEANS Procedure

Analysis Variable : AVAL Analysis Value

Parameter Code	N Obs	N	Mean	Std Dev	Minimum	Maximum
AST	670	670	25.3985075	13.2870234	9.0000000	114.0000000
CREAT	550	550	60.2605091	17.5092223	24.5200000	192.4800000
HGB	590	590	11.8327119	1.6212922	7.3000000	16.7000000

Table 4 shows the number of observations, mean and standard deviation, as well as minimum and maximum values for the three different parameters Aspartate Aminotransferase, Creatinine and Hemoglobin.

Table 5: Summary of the laboratory analysis data by gender and parameter

Sex=F

The MEANS Procedure

Analysis Variable : AVAL Analysis Value

Parameter Code	N Obs	N	Mean	Std Dev	Minimum	Maximum
AST	551	551	24.8656987	12.7349242	9.0000000	103.0000000
CREAT	466	466	57.8365880	16.6770512	24.5200000	192.4800000
HGB	493	493	11.6192698	1.5073714	7.3000000	16.7000000

Sex=M

Analysis Variable : AVAL Analysis Value

Parameter Code	N Obs	N	Mean	Std Dev	Minimum	Maximum
AST	119	119	27.8655462	15.4123681	12.0000000	114.0000000
CREAT	84	84	73.7075000	15.9267455	41.0000000	112.9200000
HGB	97	97	12.9175258	1.7491671	8.2000000	16.1000000

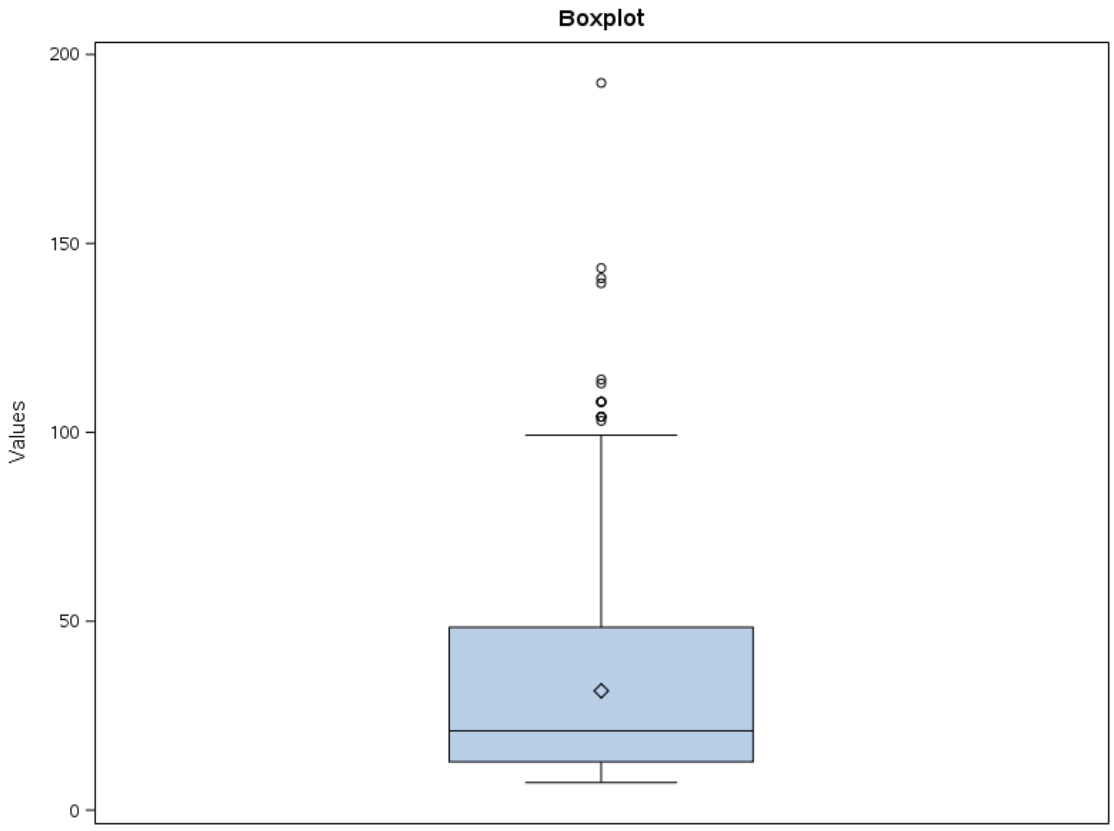
Table 5 shows the number of observations, mean and standard deviation, as well as minimum and maximum values for the three different parameters Aspartate Aminotransferase, Creatinine and Hemoglobin by gender. The mean is higher for all parameters in the male population.

BOX PLOT

The results using the normalization method are displayed as box plots using the SAS 9.4® procedure PROC SGPLOT with the VBOX statement. The VBOX statement creates box plots for the distribution of data. In this paper, the result of a specific lab test is displayed as the y-axis value. Multiple box plots can be displayed next to each other. The following descriptive measures will be displayed using by the box and whiskers:

- Median (line inside the box)
- Mean (marker inside the box)
- Q1 (25th percentile) and Q3 (75th percentile) (upper and lower fence of the box, intra-quartile range)
- Values outside the box less than or equal to 1.5 times the intra-quartile range (whiskers)
- Outliers outside the whiskers (marker points)

Figure 1: Box plot example



In Figure 1 the described characteristics of a box plot are shown.

EVALUATION AND INTERPRETATION

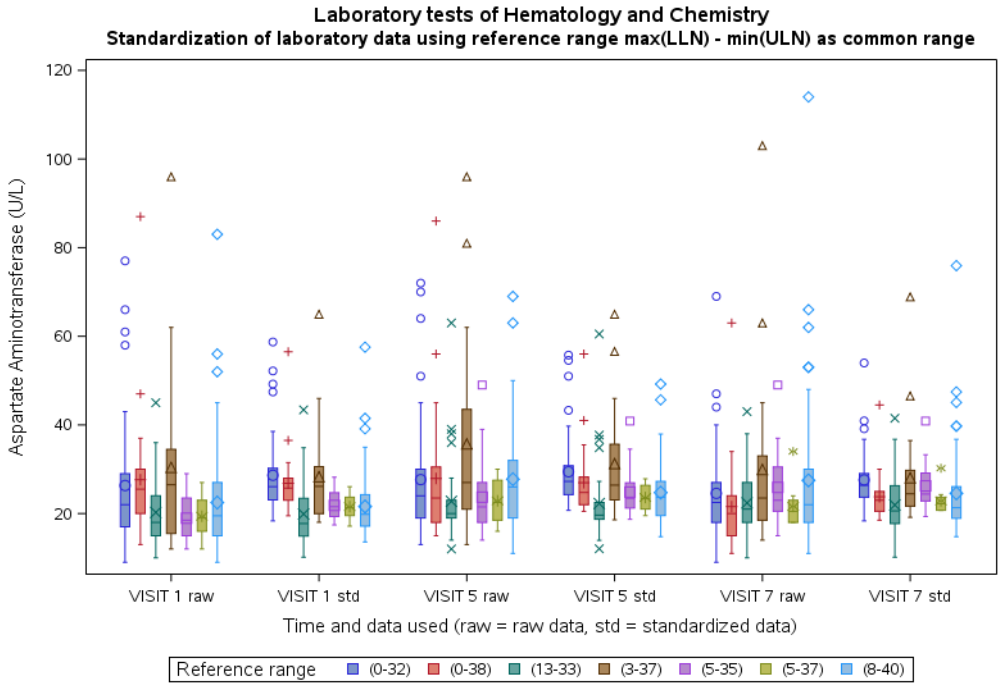
Chuang-Stein's Location-Scale model is used to analyze the data. Various methods were applied to choose the common reference ranges. The three methods used in this paper are described below:

- METHOD 1: using the maximum lower limit normal reference from all subjects and the minimum upper limit normal reference from all subjects as common reference range.
- METHOD 2: using the reference range with the maximum upper limit normal. If there are several ranges with different lower limit normal, the reference range with maximum lower limit normal within the maximum upper limit normal is used.
- METHOD 3: using the most frequent reference range for each parameter

All three methods are used to determine the common reference range for each of the three parameters. The above-mentioned model is then used to normalize the laboratory data. For the parameter Hemoglobin this is also done separately for male and female subjects. The normalized values are displayed below using box plots.

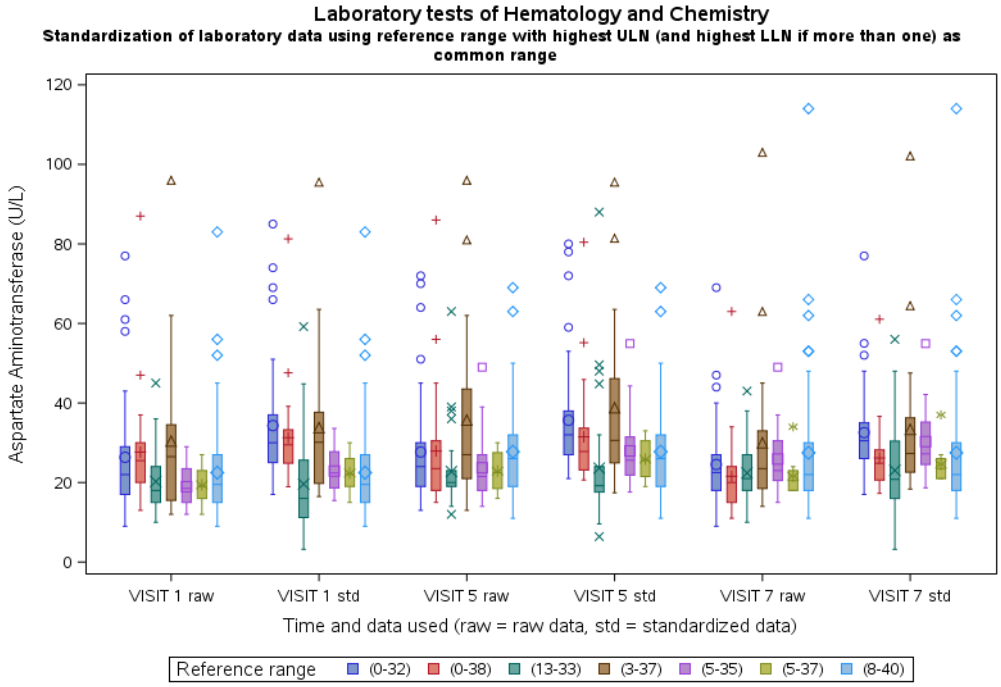
The requirement for an efficient method is that the values are comparable after normalization regardless of the laboratory and their methods used. Depending on the data, the preferable method might differ. Some examples are given below.

Figure 2: Box plot of normalized Aspartate Aminotransferase values using method 1



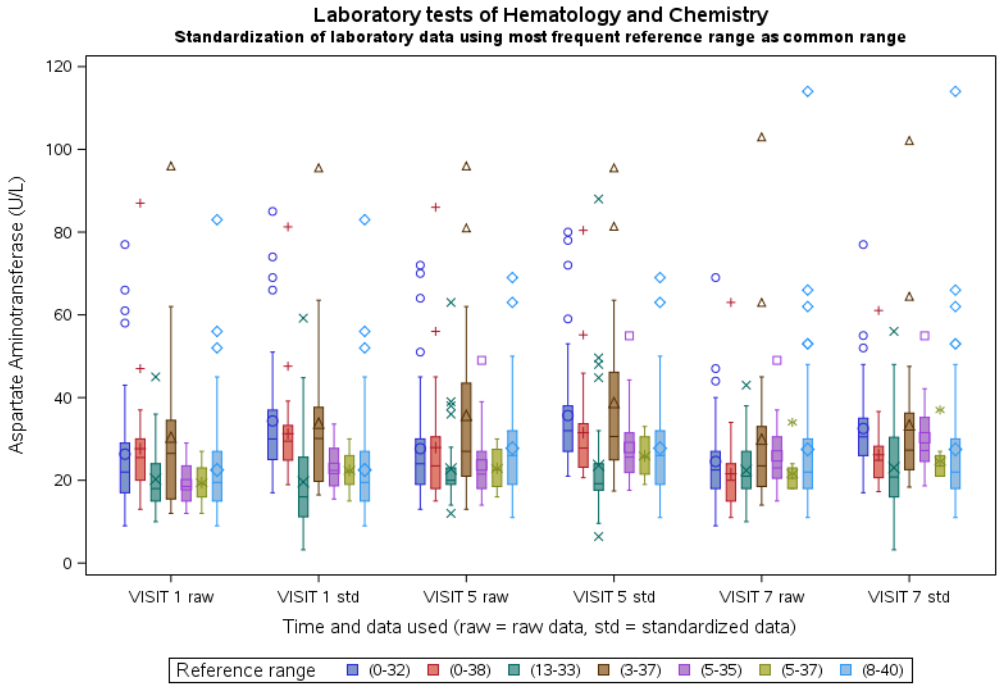
n/obs (in order of appearance): VISIT1: 51/51, 22/22, 39/39, 24/24, 20/20, 10/10, 64/64, VISIT5: 49/49, 24/24, 41/41, 24/24, 20/20, 8/8, 63/63, VISIT7: 48/48, 21/21, 35/35, 20/20, 20/20, 10/10, 57/57

Figure 3: Box plot of normalized Aspartate Aminotransferase values using method 2



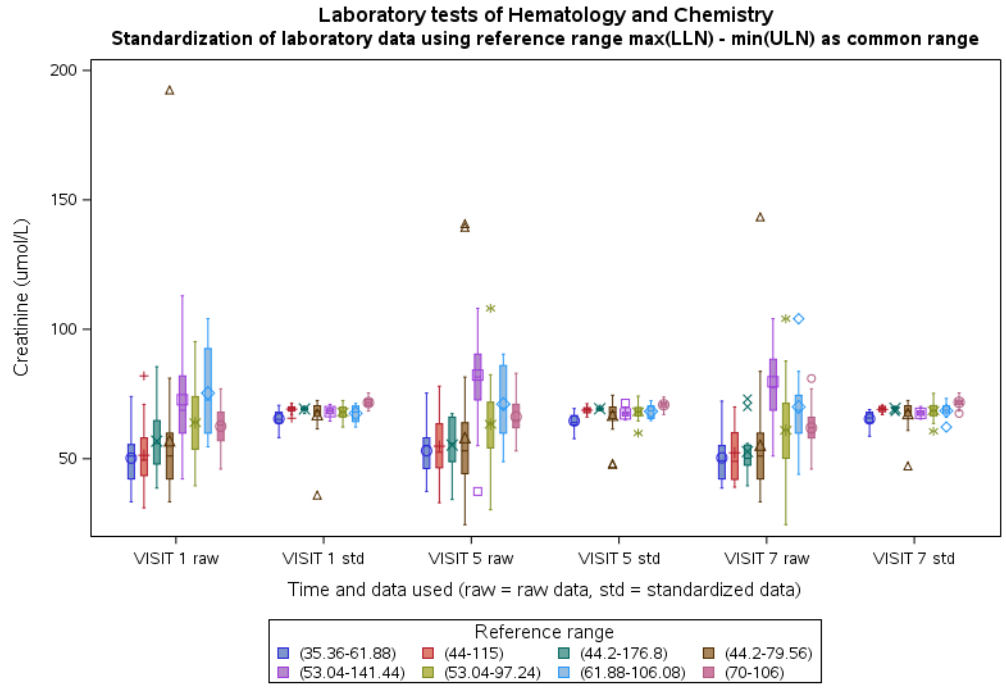
n/obs (in order of appearance): VISIT1: 51/51, 22/22, 39/39, 24/24, 20/20, 10/10, 64/64, VISIT5: 49/49, 24/24, 41/41, 24/24, 20/20, 8/8, 63/63, VISIT7: 48/48, 21/21, 35/35, 20/20, 20/20, 10/10, 57/57

Figure 4: Box plot of normalized Aspartate Aminotransferase values using method 3



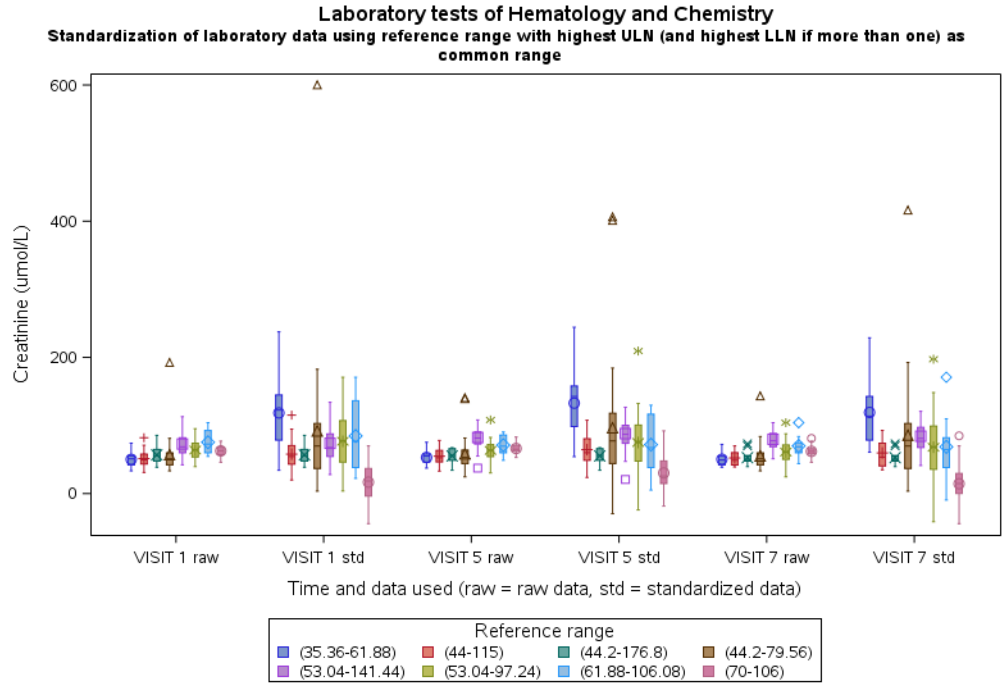
Figures 2-4 show the results for Aspartate Aminotransferase using the three different methods to choose the common reference range. The common reference ranges of methods 1, 2, and 3 are 13-32, 8-40, and again 8-40, respectively. Thus, although the calculations of the common reference ranges are different, the resulting reference ranges can be the same and consequently the normalized values. Method 2 and method 3 result in the exact same values. In method 1 the result values have a smaller range than the original values and the result values in method 2 and 3. The box plots are smaller. The outliers are also closer to the box. For method 2 and 3 the box plots are not significantly changed before and after the normalization. It can be predicated that for the available data of parameter Aspartate Aminotransferase using method 1 is the most suitable approach.

Figure 5: Box plot of normalized Creatinine values using method 1



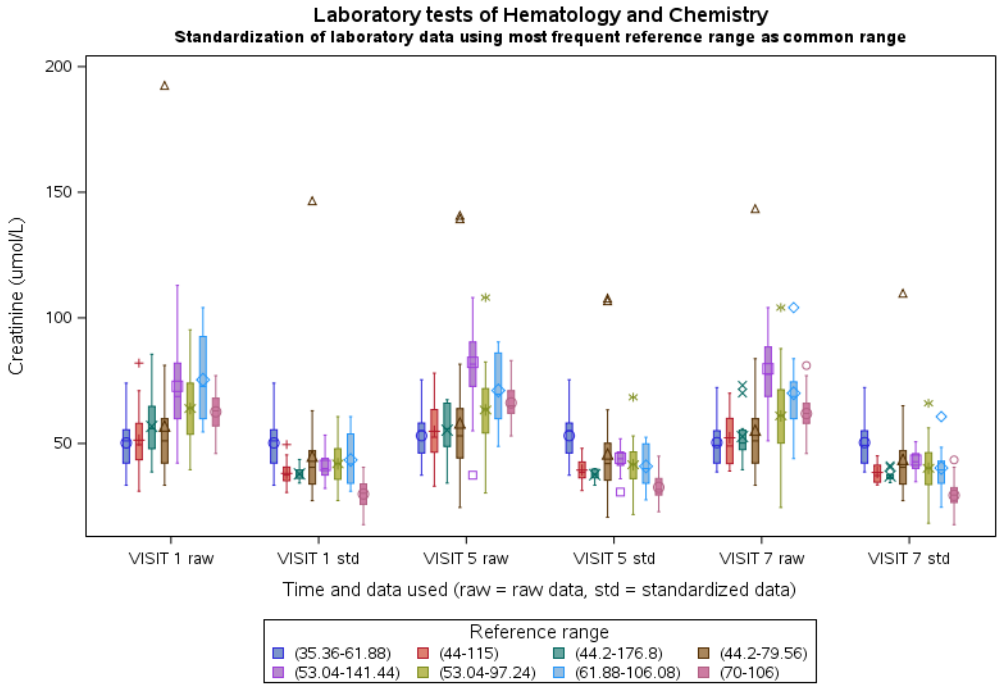
n/obs (in order of appearance): VISIT1: 31/31, 32/32, 12/12, 30/30, 24/24, 29/29, 10/10, 21/21, VISIT5: 33/33, 32/32, 14/14, 28/28, 24/24, 29/29, 10/10, 21/21, VISIT7: 27/27, 25/25, 12/12, 30/30, 20/20, 26/26, 9/9, 21/21

Figure 6: Box plot of normalized Creatinine values using method 2



n/obs (in order of appearance): VISIT1: 31/31, 32/32, 12/12, 30/30, 24/24, 29/29, 10/10, 21/21, VISIT5: 33/33, 32/32, 14/14, 28/28, 24/24, 29/29, 10/10, 21/21, VISIT7: 27/27, 25/25, 12/12, 30/30, 20/20, 26/26, 9/9, 21/21

Figure 7: Box plot of normalized Creatinine values using method 3

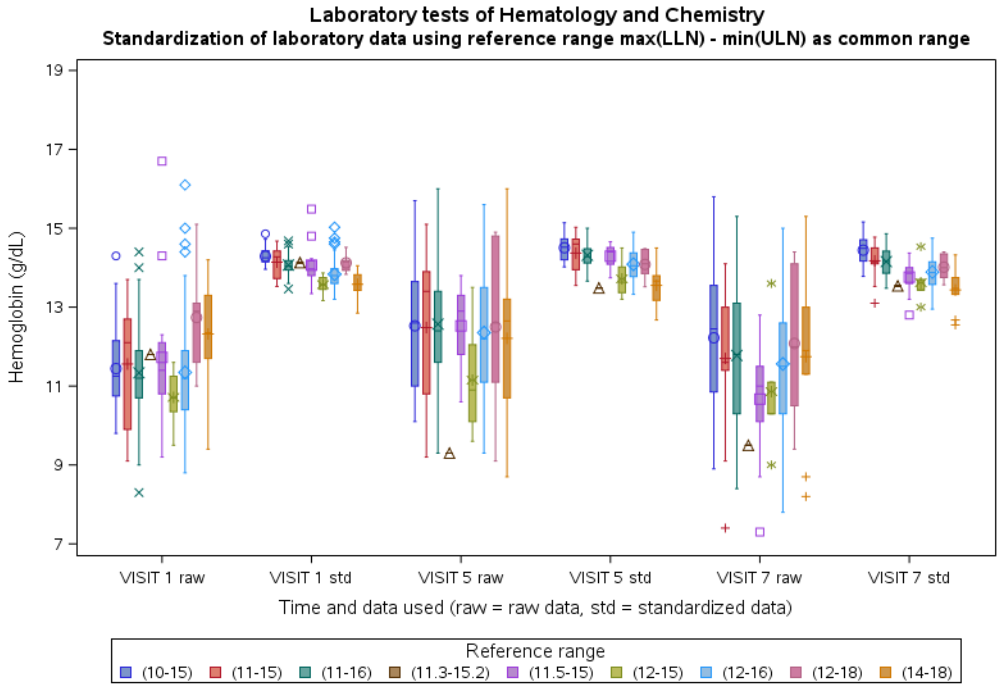


Figures 5-7 show the results for Creatinine using the three different methods to choose the common reference range. The common reference ranges of methods 1, 2, and 3 are 70-61.88, 44.2-176.8, and 35.36-61.88, respectively. In method 1 the normalized values have only a small range, the box plots are a lot smaller than before normalization. When looking at the common reference ranges the LLN is higher than the ULN which is questionable to use for normalization since this is not possible in real data. In method 2 the opposite occurs, the normalized values have a higher range, the box plots get bigger and the means of the groups for each visit have a greater variation. Moreover, this method leads to some negative standardized values which is not desirable for a normalization process. If a low assay value is normalized with respect to a reference range which is wider than the original reference range and with a lower limit of normal close to zero, a negative normalized value can occur. Chuang-Stein suggested to replace a negative normalized value with zero, whereas Karvanen et al. proposed that negative normalized values are an indication for an unsuitable normalization method.
Method 3 shows reasonable results, the variation of the normalized values is smaller than in the original laboratory values. The means of the different groups get closer with each visit and lie a bit below the means of the original means. This method is preferable as normalization method for Creatinine. Another possibility for this parameter would be to apply the scale model.

When applying method 2 for Creatinine, there are four obvious statistical outliers (one for Visit 1, two for visit 5 and one for visit 7). After normalization two subjects have normalized values higher than 400. Subject 144 has a value higher than 400 at all three visits and subject 498 has a value higher than 400 at Visit 5. How these outliers can arise is exemplarily calculated for subject 144 at visit 1:

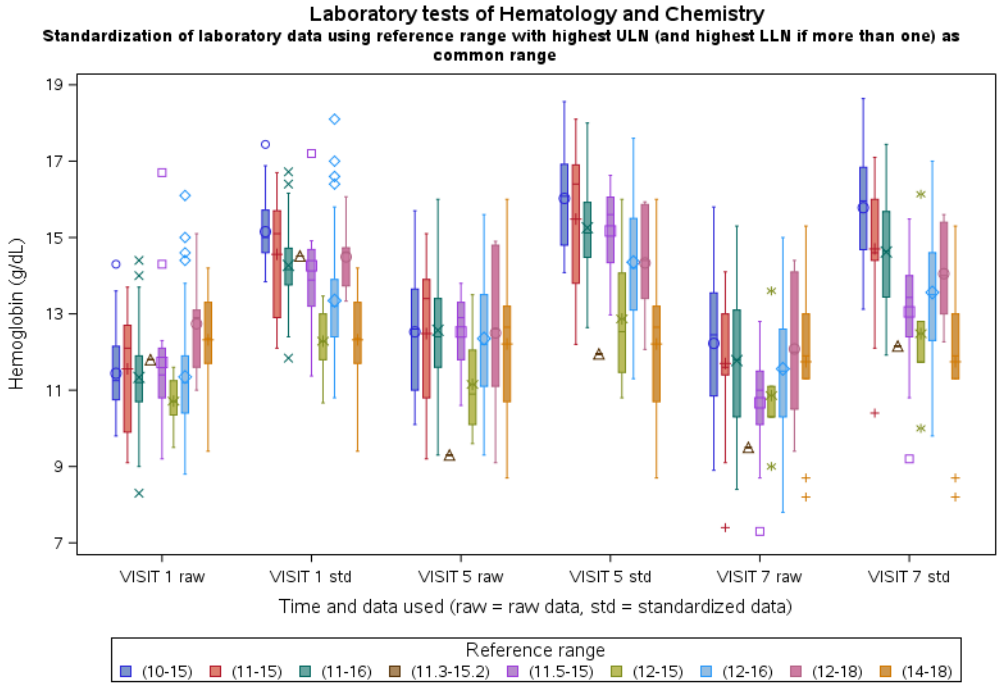
$$value_{std} = (192.48 - 44.2) * \frac{176.8 - 44.2}{79.56 - 44.2} + 44.2 = 600.25$$

Figure 8: Box plot of normalized Hemoglobin values using method 1



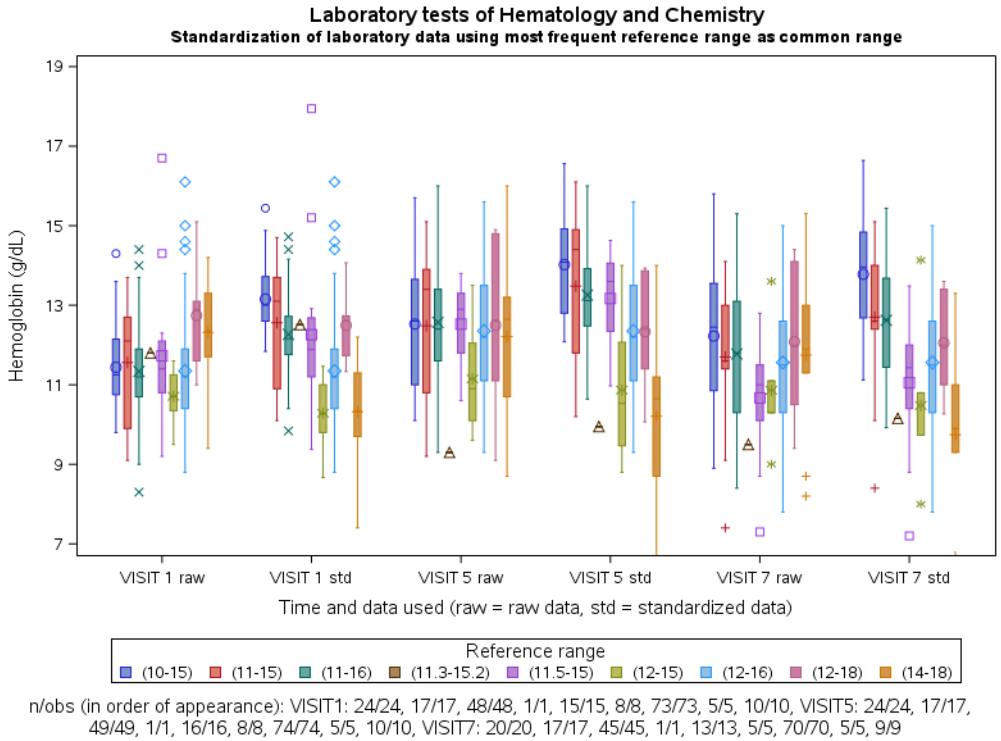
n/obs (in order of appearance): VISIT1: 24/24, 17/17, 48/48, 1/1, 15/15, 8/8, 73/73, 5/5, 10/10, VISIT5: 24/24, 17/17, 49/49, 1/1, 16/16, 8/8, 74/74, 5/5, 10/10, VISIT7: 20/20, 17/17, 45/45, 1/1, 13/13, 5/5, 70/70, 5/5, 9/9

Figure 9: Box plot of normalized Hemoglobin values using method 2



n/obs (in order of appearance): VISIT1: 24/24, 17/17, 48/48, 1/1, 15/15, 8/8, 73/73, 5/5, 10/10, VISIT5: 24/24, 17/17, 49/49, 1/1, 16/16, 8/8, 74/74, 5/5, 10/10, VISIT7: 20/20, 17/17, 45/45, 1/1, 13/13, 5/5, 70/70, 5/5, 9/9

Figure 10: Box plot of normalized Hemoglobin values using method 3



Figures 8-10 show the results for Hemoglobin using the three different methods to choose the common reference range. The common reference ranges of methods 1, 2, and 3 are 14-15, 14-18, and 12-16, respectively. In method 1, the variation in the normalized values is smaller than in the original laboratory values and the means of the different groups get closer with each visit. However, a noteworthy aspect is that the common reference range is really narrow (14-15) and that this has already led to a shrunken range of standardized values. It is questionable if such a small reference range would appear in real data. The results in method 2 and method 3 are comparable. The ranges in the standardized values are similar to the ranges in the original values. In method 2 the means lie a little bit above the means in the original values. In both methods (2 and 3) the box plots are not significantly different before and after the normalization, so it does not lead to a harmonization in the data.

Hemoglobin is perhaps the best-known laboratory parameter where gender differences appear. Therefore, we apply the normalization approach using the three different methods separated by gender.

Figure 11: Box plot of normalized Hemoglobin values of female subjects using method 1

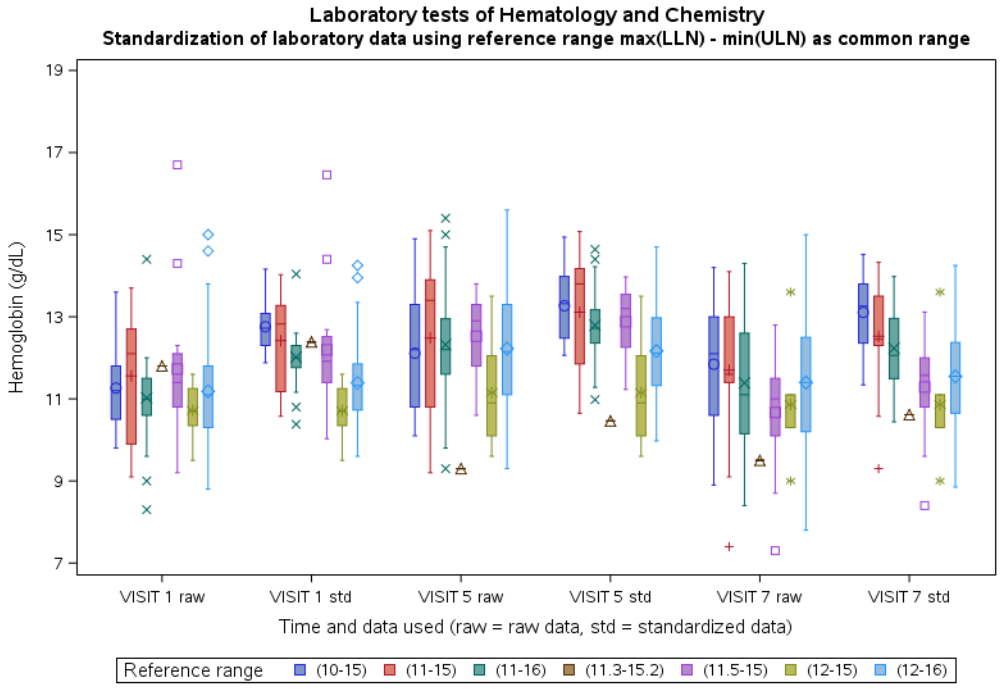


Figure 12: Box plot of normalized Hemoglobin values of female subjects using method 2

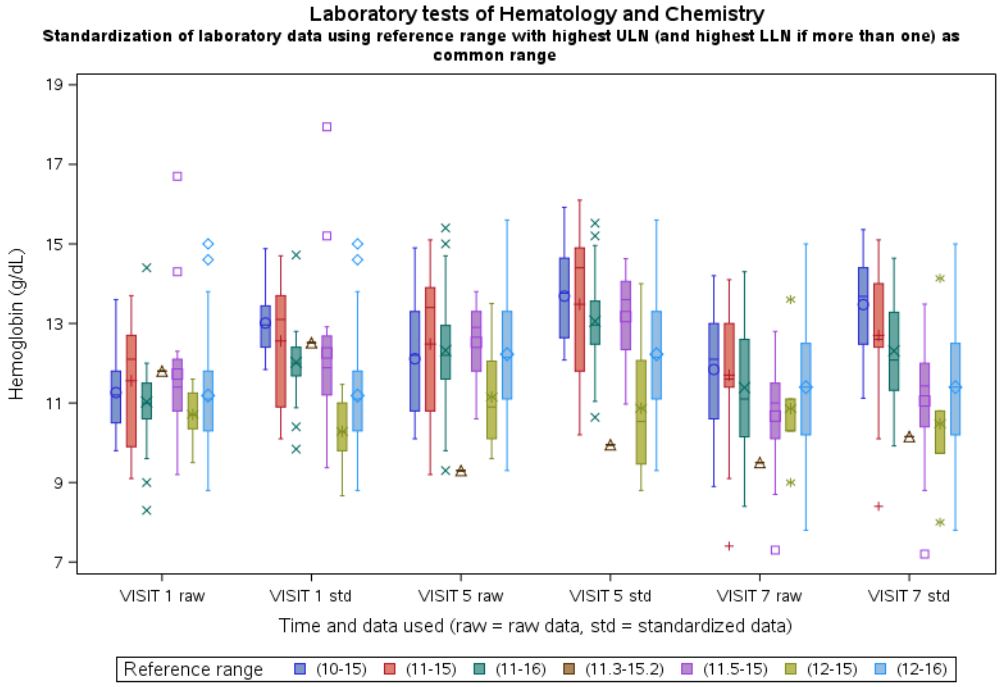
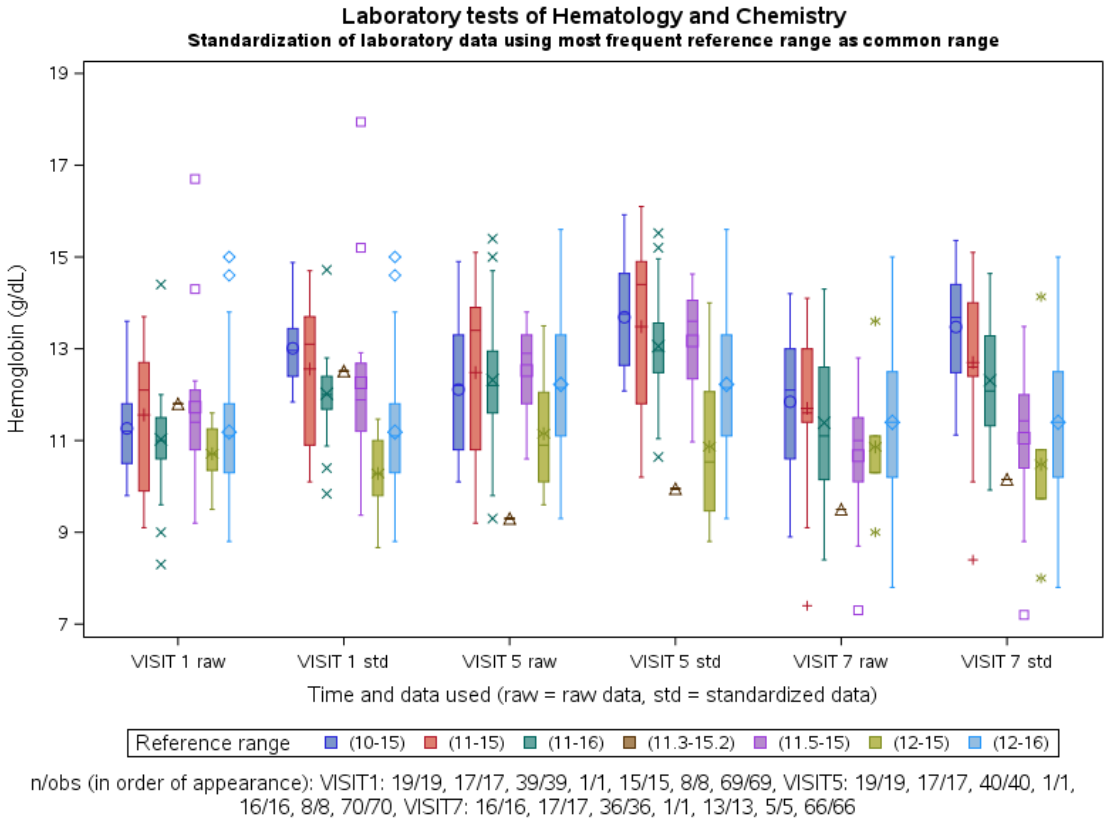


Figure 13: Box plot of normalized Hemoglobin values of female subjects using method 3



Figures 11-13 show the results for Hemoglobin in female subjects using the three different methods to choose the common reference range. The common reference ranges of methods 1, 2, and 3 are 12-15, 12-16, and again 12-16, respectively. As for Aspartate Aminotransferase the resulting common reference ranges in method 2 and 3 are the same, resulting also in the exact same normalized values. In method 1 the normalized values have slightly smaller ranges than the original values and the normalized values in method 2 and 3. The outliers are in comparison closer to the box. However, the differences are very small. Moreover, the distances of the means are similar compared to the original assay values. In total, the differences between the original and the normalized values are marginal. In method 2 and 3 the box plots are also not significantly different before and after normalization.

Figure 14: Box plot of normalized Hemoglobin values of male subjects using method 1

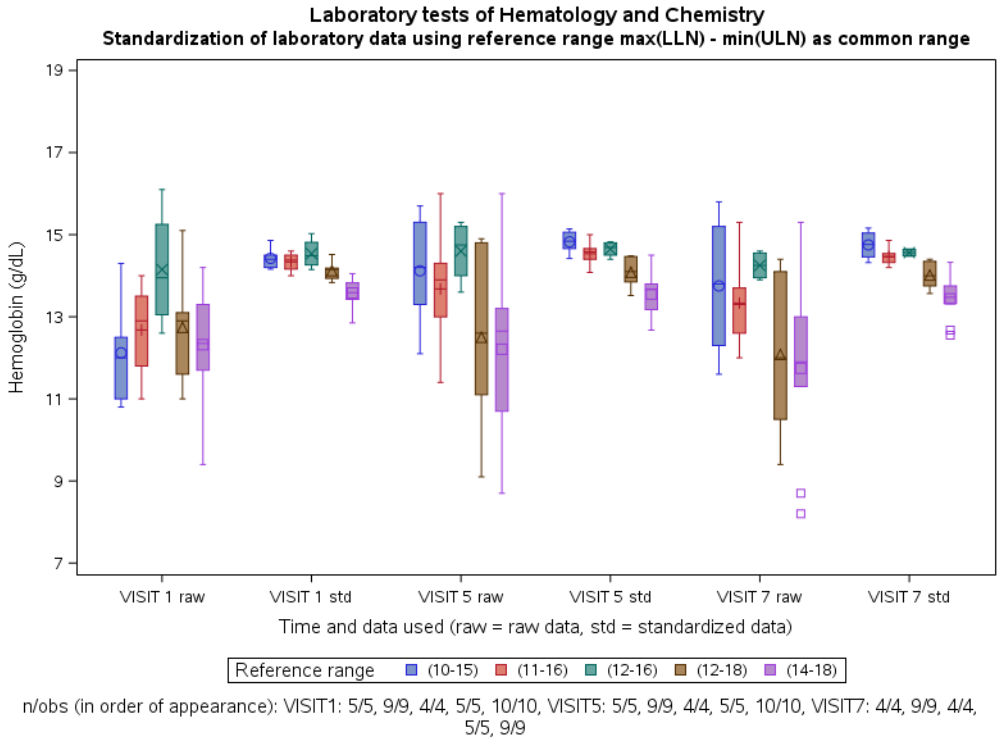


Figure 15: Box plot of normalized Hemoglobin values of male subjects using method 2

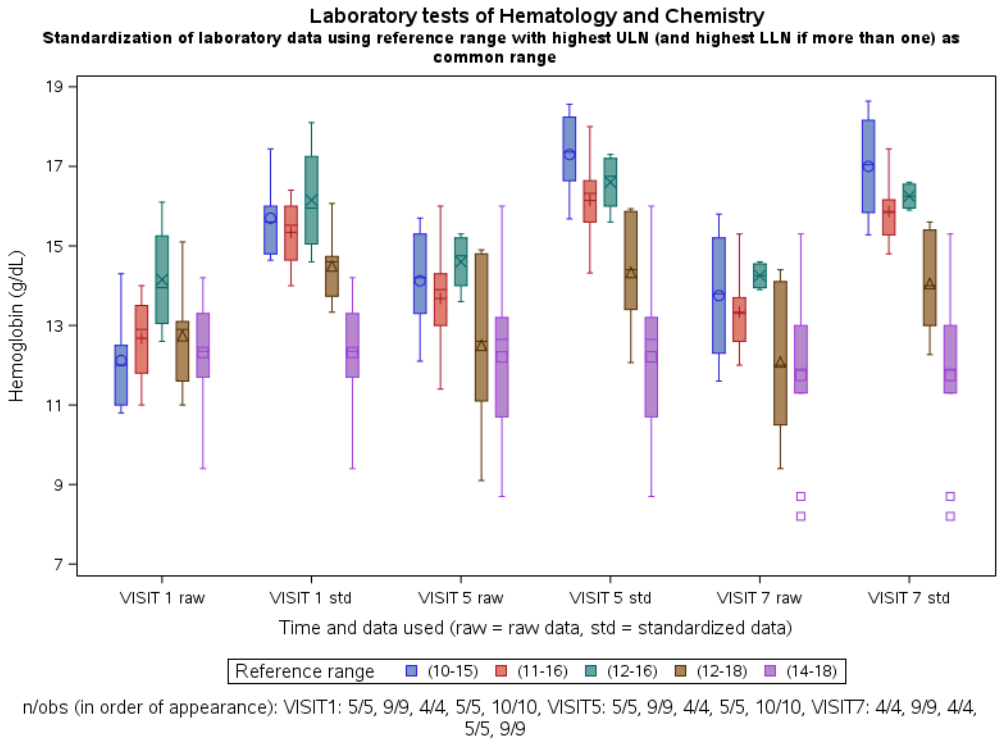
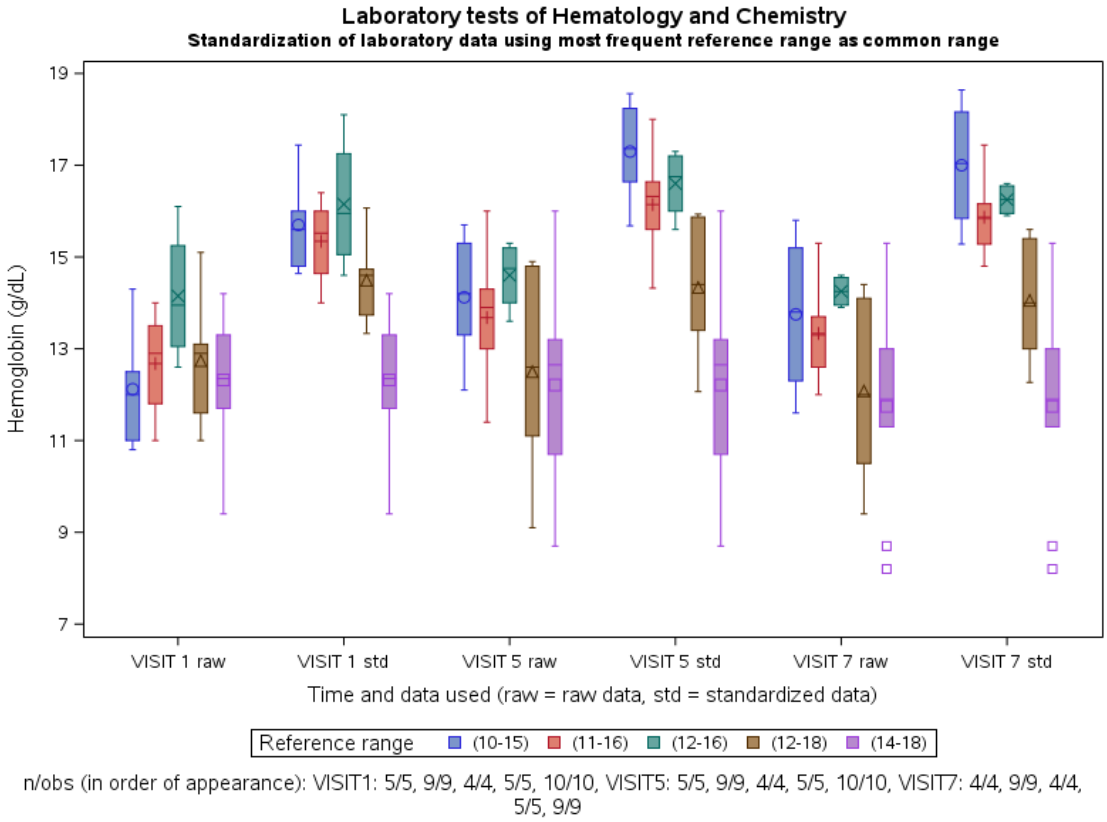


Figure 16: Box plot of normalized Hemoglobin values of male subjects using method 3



Figures 14-16 show the results in Hemoglobin for male subjects using the three different methods to choose the common reference range. The common reference ranges of methods 1, 2, and 3 are 14-15, 14-18, and again 14-18, respectively. In method 1, the variation in the normalized values is smaller than the variation of the original laboratory values and the means in the different groups get closer after each visit. However, a noteworthy fact is that the common reference range is really narrow (14-15) and has already led to a shrunken range of standardized values. It is questionable whether such a small reference range would occur in real data. As you can see above for female subjects, the resulting common reference ranges in method 2 and 3 are the same, resulting in exactly the same normalized values. The box plots are not significantly different before and after normalization.

CONCLUSION

Chuang-Stein's location-scale model is used to normalize the existing laboratory data for the three parameters Aspartate Aminotransferase, Creatinine and Hemoglobin. To apply this normalization model, a common reference range has to be selected. This is done by using the above-mentioned methods. The most suitable approach to choose the common reference range depends on the parameter and is always different. Aspartate Aminotransferase method 1 shows reasonable results as the variation in the normalized values is smaller and the means in the various groups get closer. Method 3 is the most suitable method for analyzing Creatinine because the means get closer and the variation of the normalized data gets smaller. For Creatinine it might also be worth considering to apply another normalization model. Method 1 and 2 are unsuitable for analyzing Creatinine data in this paper. By applying method 1 the lower limit of normal is greater than the upper limit of normal for the common reference range, which is not possible in real data. Using method 2 leads to negative values, which also indicates that the normalization method is inappropriate. For Hemoglobin, method 1 seems to be the most appropriate method to choose the common reference range, but one must be critical as the resulting reference range is really small and this does not reflect reality. Since Hemoglobin is known to differ according to gender, an analysis by gender was also done for this parameter. In the box plots for the male patients the average values are higher than the average values for female patients. This applies to

the values before and after normalization. For female patients, the box plots in all three methods are similar since the chosen common reference ranges are quite similar or exactly the same in method 2 and method 3. For male patients, the common reference ranges are equal in method 2 and method 3 as well. Method 1 has a small chosen range, which leads to narrow box plots and it is questionable whether such small ranges would occur in real data. In such cases it can be discussed if the normalization of the values has any advantages compared to using the original data values.

As summarized above, the results in the location-scale model differ for each parameter. The normalization model is combined with three different methods in order to determine a common reference range. Depending on the parameter, the outcome of each method varies a lot and it is not possible to choose a method that can be used in all analyses.

Normalizing laboratory data can be a solution to pool data from different laboratories with different reference ranges. However, it is not an ideal option to analyze laboratory data. As far as possible differences in the laboratory reference ranges should be avoided for patients with equal characteristics (e.g. gender and age). One possibility to reduce this issue is using a central laboratory which has already become a common practice. Nevertheless, there are still some issues that remain. Differences regarding gender or age will still persist. If sample sizes are sufficient, a solution might be to analyze the data by laboratory or by gender or age. Moreover, if an assay procedure was changed during a study, a centralized laboratory does not solve this problem. When pooling different studies, different centralized laboratories may be involved and the issue of different reference ranges may still exist.

As shown in this paper, the choice of using the normalization model and common reference range is very important. All laboratory parameters have their clinical interpretations and statistical properties. The preferred normalization model as well as the most suitable choice of the common reference range depend on the laboratory parameter and cannot be generalized. It is very important to have a deep insight into the data and to investigate it carefully because normalization should be a well-considered process.

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