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Measurement in Health and Disease

Reference Intervals and Centile Charts

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Normal range or reference interval

What values are measurements on normal, healthy people likely to be?

Difficulties:

Who is 'normal'?

What is a range?

Normal biology or Normal distribution?

Normal range or reference interval

What values are measurements on normal, healthy people likely to be?

Difficulties:

Who is 'normal' anyway?

Usually say that normal people are the apparently healthy members of the local population.

We draw a representative sample of these and make the measurement on them.

Normal range or reference interval

What values are measurements on normal, healthy people likely to be?

Difficulties:

If we use the range of the observations, the difference between the two most extreme values, if we carry on sampling we will eventually find observations outside it, and the range will get bigger and bigger.

Use a range between two quantiles, usually the 2.5 centile and the 97.5 centile, which is called the **normal range**, **95% reference range** or **95% reference interval**.

This leaves 5% of normals outside the 'normal range', which is the set of values within which 95% of measurements from apparently healthy individuals will lie.

Normal range or reference interval

What values are measurements on normal, healthy people likely to be?

Difficulties:

Confusion between 'normal' as used in medicine and 'Normal distribution' as used in statistics.

Misleading approaches which say that all data which do not fit under a Normal curve are abnormal.

The term 'reference interval' has the advantage of avoiding this confusion.

However, the most commonly used method of calculation rests on the assumption that the variable follows a Normal distribution.

The standard deviation method

In general, most observations fall within two standard deviations of the mean.

For a Normal distribution, 95% of observation are within these limits with 2.5% below the lower limit and 2.5% above the upper limit.

(To be more precise, we should say ±1.96 rather than 2, but the difference is tiny.)

If we estimate the mean and standard deviation of data from a Normal population we can estimate the reference interval as $\bar{x} - 2s$ to $\bar{x} + 2s$.

The standard deviation method

```
FEV1 measurements (litres) for 57 male medical students
2.85  3.19  3.50  3.69  3.90  4.14  4.32  4.50  4.80  5.20
2.85  3.20  3.54  3.70  3.96  4.16  4.44  4.56  4.80  5.30
2.98  3.30  3.54  3.70  4.05  4.20  4.47  4.68  4.90  5.43
3.04  3.39  3.57  3.75  4.08  4.20  4.47  4.70  5.00
3.10  3.42  3.60  3.78  4.10  4.30  4.47  4.71  5.10
3.10  3.48  3.60  3.83  4.14  4.30  4.50  4.78  5.10
```

The observations appear to follow a distribution which is close to the Normal.
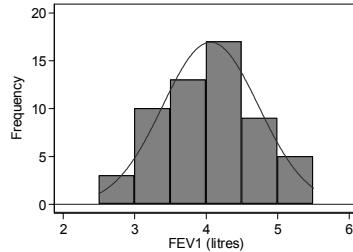
The standard deviation method

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3.10  3.48  3.60  3.83  4.14  4.30  4.50  4.78  5.10
```

We have 57 observations, mean 4.06 and standard deviation 0.67 litres.

The observations appear to follow a distribution which is close to the Normal.

The reference interval is thus 4.06 – 2×0.67 = 2.7
to 4.06 + 2×0.67 = 5.4 litres.

Only one student (2%) is outside these limits.

The standard deviation method

We have 57 observations, mean 4.06 and standard deviation 0.67 litres.

The reference interval is thus 4.06 – 2×0.67 = 2.7 to 4.06 + 2×0.67 = 5.4 litres.

As the observations are assumed to be from a Normal distribution, standard errors and confidence intervals for these limits are easy to find.

Standard error for 95% reference limits are approximately equal to $s\sqrt{3}/n$.

Standard error is 0.67×√3/57 = 0.15. Hence the 95% confidence intervals for these limits are 2.7 ± 1.96× 0.15 and 5.4 ± 1.96×0.15, i.e. from 2.4 to 3.0 and 5.1 to 5.7 litres.
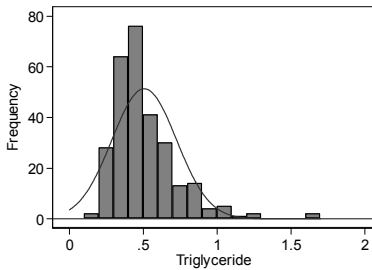
Data which do not follow a Normal distribution

Serum triglyceride measurements in cord blood from 282 babies and Normal curve with same mean and standard deviation:
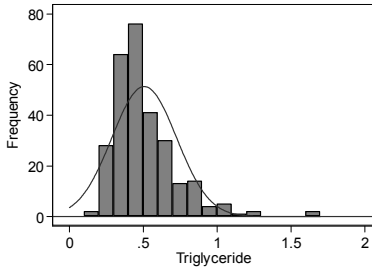
The data are highly skewed, and we cannot use the Normal method directly.

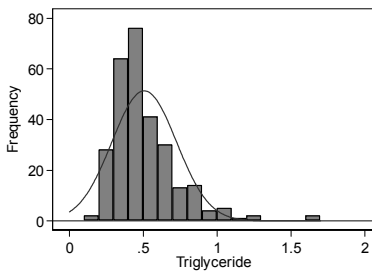**Data which do not follow a Normal distribution**

Serum triglyceride measurements in cord blood from 282 babies and Normal curve with same mean and standard deviation:

If we did, the lower limit would be 0.07, well below any of the observations.

The upper limit would be 0.94, greater than which are 5% of the observations.

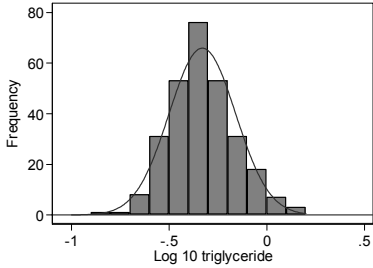**Data which do not follow a Normal distribution**

Serum triglyceride measurements in cord blood from 282 babies and Normal curve with same mean and standard deviation:

It is possible for such data to give a negative lower limit.

Data which do not follow a Normal distribution

Log_{10} transformed serum triglyceride measurements in cord blood from 282 babies and Normal curve with same mean and standard deviation:

We now have a symmetrical distribution which is a good fit to the Normal.

We can apply the standard deviation method.

Data which do not follow a Normal distribution

Log_{10} transformed serum triglyceride measurements in cord blood from 282 babies:

The mean and standard deviation are –0.331 and 0.171.

The lower limit in the log transformed data is
$$-0.331 - 2 \times 0.171 = -0.673.$$

If we antilog this, we get a triglyceride level equal to 0.21.

2.1% of observations are below this.

The upper limit is –0.331 + 2×0.171 = 0.011.

If we antilog this, we get a triglyceride level equal to 1.02.

2.5% of observations are above this.

The fit to the log transformed data is excellent.

Data which do not follow a Normal distribution

Log_{10} transformed serum triglyceride measurements in cord blood from 282 babies:

For the standard error of the reference limit we have
$0.171 \times \sqrt{3/282} = 0.0176$.

The 95% confidence intervals are thus
$$-0.673 \pm 1.96 \times 0.0176 \quad \text{and} \quad 0.011 \pm 1.96 \times 0.0176$$

 –0.707 to –0.639 and –0.023 to 0.045.

Transforming back, we get 0.20 to 0.23 and 0.95 to 1.11.

Direct estimation of the centiles

Some authors have advocated the estimation of the centiles directly, without any distributional assumptions.

This is an attractive idea.

Rank the observations and find the point below which 2.5% of the observations fall.

For the 282 triglycerides, we find the observation whose rank is 0.025×(282 + 1) = 7.08.

The required quantile will be between the 7th and 8th observations. The 7th is 0.21, the 8th is 0.22 so the 2.5 centile would be estimated by
$$0.21 + (0.22 - 0.21)×(7.08 - 7) = 0.21.$$

Similarly the 97.5 centile is 1.04.

Direct estimation of the centiles

This approach gives an unbiased estimate whatever the distribution.

The log transformed triglyceride would give exactly the same results.

Note that the Normal theory limits from the log transformed data are very similar.

Direct method: 95% reference interval = 0.21 to 1.04.

Standard deviation method:
95% reference interval = 0.21 to 1.02.

Direct estimation of the centiles

Confidence interval is estimated by a direct method which tells us which rank the observations at the lower and upper confidence limits will be.

For the triglyceride data, for the lower limit of the reference range the 95% confidence interval will be from the 2nd to the 13th observations.

The second observation is 0.16 and the 13th is 0.26, so the 95% confidence interval for the lower reference limit is 0.16 to 0.26.

The corresponding calculation for the 97.5 centile gives the 270th to the 281st observation, giving a 95% confidence interval for the upper reference limit of 0.96 to 1.64.

Direct estimation of the centiles

Direct method: 95% reference interval = 0.21 to 1.04.

95% confidence intervals 0.16 to 0.26 and 0.96 to 1.64.

Standard deviation method:
        95% reference interval = 0.21 to 1.02.

95% confidence intervals 0.20 to 0.23 and 0.95 to 1.11.

The standard deviation method gives very similar estimates with much narrower confidence intervals.

The direct method of estimating centiles in long tails is relatively imprecise.

Centile charts

Reference range for different values of some other variable, usually age.

Centile chart of fetal head circumference by gestational age (Schwarzler *et al.*, 2004)
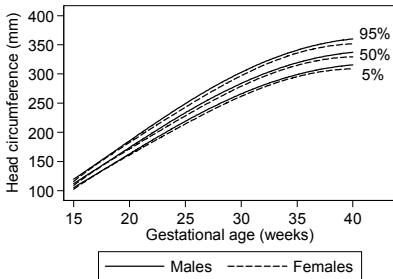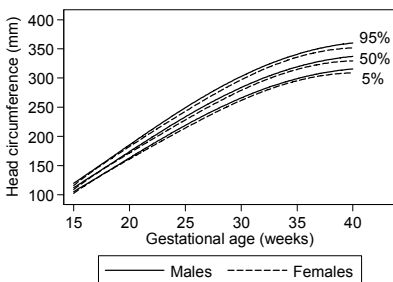

Schwarzler P, Bland JM, Holden D, Campbell S, Ville Y. (2004) Sex-specific antenatal reference growth charts for uncomplicated singleton pregnancies at 15-40 weeks of gestation. *Ultrasound in Obstetrics & Gynaecology* 23, 23-29.

Centile charts

90% reference range for fetal head circumference.

This enables us to see at the glance the range for fetuses of any gestational age.


Schwarzler P, Bland JM, Holden D, Campbell S, Ville Y. (2004) Sex-specific antenatal reference growth charts for uncomplicated singleton pregnancies at 15-40 weeks of gestation. *Ultrasound in Obstetrics & Gynaecology* 23, 23-29.

Centile charts

Like any reference range, the centile chart is estimated from data on an apparently normal set of subjects.

It was important to exclude any extra measurements on fetuses made because there was concern about the development of the pregnancy, as these would produce a bias in the estimate.
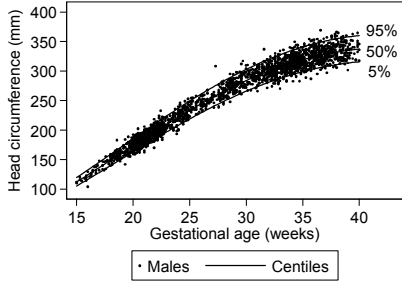
Centile charts

Several different methods have been used to generate centile charts, but the best is due to Altman (1993).

First we carry out regression with head circumference as the outcome (or dependent) variable and gestational age as the predictor (explanatory or independent) variable.

We use a regression method which enables us to fit curves rather than straight lines.
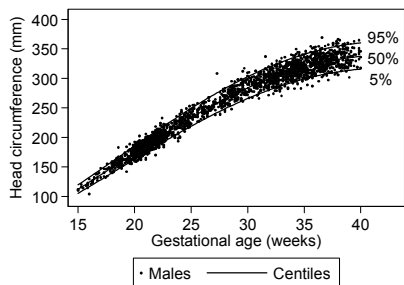
This enables us to estimate the mean head circumference for any gestational age.

The problem is that the standard deviation can vary with gestational age, too.

Altman DG. (1993) Construction of age-related reference centiles using absolute residuals. *Stat Med.* **12**, 917-924.

Centile charts

The problem is that the standard deviation can vary with gestational age, too.

Centile charts

The problem is that the standard deviation can vary with gestational age, too.

We can estimate the standard deviation from the residuals, the differences between the observed values and those predicted by the regression equation.

Altman (1993) showed that, provided the residuals for any given gestational age followed a Normal distribution, the absolute value of the residual, without the + or – sign, can be used to estimate the standard deviation.

Centile charts

Do regression with the absolute residual as the outcome variable and gestational age as the predictor.

The standard deviation is given by the predicted absolute residual multiplied by $\sqrt{\pi/2}$.

We now have equations to predict the mean and the standard deviation from the gestational age.

We can use these to calculate the centiles as we did for a simple reference range.

Centile charts

We now have equations to predict the mean and the standard deviation from the gestational age.

We can use these to calculate the centiles as we did for a simple reference range.