Diplomarbeit

The Neutrophil-to-Lymphocyte ratio in Pregnancy

Eingereicht von

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Doktor der gesamten Heilkunde

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an der

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Die Arbeit wurde verfasst an der

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Graz, den 03.08.2017

I. Statutory Declaration

I declare that I have authored this thesis independently, that I have not used other than the declared sources / resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

Graz, den 03.08.2017

Benedikt Heitmeir eh.

II. Acknowledgement

This thesis was performed within a research exchange program of the Medical University Graz and the University of New South Wales, Australia.
I want to thank Professor Craig McLachlan for making this thesis possible and for supervising my work.

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Many thanks to my dear colleagues and staff at the clinic and office for the comfortable working atmosphere, the discussions during the coffee breaks and the fun we had outside the world of work. I also want to thank all my friends and family for supporting me during this endeavour.

III. Contents

IV. Zusammenfassung

Diese Arbeit wurde an der University of New South Wales, Australien im Rahmen eines Austauschprogrammes mit der Medizinischen Universität Graz angefertigt. Die verwendeten Datensätze wurden an den Krankenhäusern der Albury Wodonga Health zusammengetragen.

Die Arbeit befasst sich mit der Untersuchung des Verhältnisses von neurophilen Granulozyten zu Lymphozyten (NLR) und deren Veränderung im Laufe einer Schwangerschaft. Im Rahmen der Studie wurden eine Reihe von weiteren Parametern und Blutwerte erhoben. Auch diese wurden auf eventuelle Abhängigkeiten oder Muster überprüft. Die erhobenen mütterlichen Parameter waren: BMI, Körpergröße, Fundusstand, Alter, Gewicht vor der Schwangerschaft und Anzahl der Schwangerschaften.
Zusätzliche wurden kindlichen Einflussfaktoren erhoben: Größe, Gewicht und Kopfumfang bei Geburt.
Es zeigt sich, dass das Verhältnis von neutrophilen Granulozyten und Lymphozyten im Laufe der Schwangerschaft von 2.83±1.57 im ersten Trimester auf 4.77±3.37 im dritten Trimester ansteigt, so dass hier ein Zusammenhang gezeigt werden konnte.
Es konnte ebenso gezeigt werden, dass keine weitere Abhängigkeit zwischen der NLR und den anderen erhobenen Faktoren besteht. Die Zunahme der NLR Werte im Laufe der Schwangerschaft deutet auf inflammatorische Prozesse hin, die während der Schwangerschaft ablaufen.

V. Abstract

This paper evaluates the development of the Neutrophil to Lymphocyte Ratio and a number of influencing factors in pregnancy. It was established that NLR increases during pregnancy from 2.83±1.57 in the first trimester to 4.77±3.37 in the third trimester. Thus it could be shown that the NLR increases during pregnancy. Further investigations with the additional parameters collected during this study. The focus was to determine which additional factors also influence the NLR value in or during the pregnancy such as maternal age, maternal BMI, maternal height, fundal height and pre-pregnancy weight. In addition, neonatal parameters such as birthweight, length at birth and head circumference were examined for associations with the NLR. No significant dependencies could be found there.

The increase of the NLR value during pregnancy is the result of inflammatory processes in pregnancy.

VI. Abbreviations

AWH	Albury-Wodonga Health
BMI	Body mass index
FBC	Full blood count
FBE	Full blood examination
GTT	Glucose Tolerance Test
HCG	Human chorionic gonadotropin
NLR	Neutrophil-to-Lymphocyte ratio
SFH	Symphysis fundal height
WCC	White cell count

1. Introduction and Literature review

Neutrophil granulocytes are the most important part in the innate unspecific immune response (1). They are the most numerous of the white cells. They are the predominant antimicrobial force in the human body with their ability to phagocyte bacteria and induce inflammation. Furthermore, they also interact with the specific immunity and play a major part in its activation (1). Since the body of the pregnant woman adapts to the changed circumstances the neutrophil granulocyte frequency changes too. There is a notable increase in the frequency of the neutrophil granulocytes in the blood during the course of the pregnancy. This is even more important since the there is an increase in the total blood volume, which is especially pronounced during the last trimester of pregnancy (2).

Lymphocytes are another important part of the immune system. They are only found in vertebrate animals. They consist of 3 subgroups, T-cells, B-cells and natural killer cells. T-cells are mainly active in the cell-mediated immune response. B-cells play a major role in the humoral immune system. The natural killer cell is a part of the innate immune system and plays a major role in the defence against tumour cells and virally infected cells. Since lymphocytes have a long lifespan their numbers show smaller changes compared to neutrophil granulocytes.

There is limited research that pertains to the Neutrophil-to-Lymphocyte Ratio (NLR) during normal pregnancy. NLR combines the absolute number of neutrophils with the absolute number of lymphocytes in the peripheral blood. NLR is a parameter for systemic inflammation currently not used in routine clinical practice or in pregnancy assessments. There is limited data describing the normal range in an uneventful pregnancy. Because pregnancy and parturition are influenced by inflammation there has been some research looking at NLR and certain complications of pregnancy (3-6). The purpose of this study was to determine a normal range of NLR values in uncomplicated pregnancies. There is a wide variety of factors that can influence either the number of neutrophils granulocytes or the number of lymphocytes in the blood and thus the NLR, selection of these factors was also graphically examined (1,2,7).

NLR is a marker of systemic inflammation. Because systemic inflammation plays a major role in the diagnosis and prognosis of cancer, NLR has been studied extensively in solid cancers. Templeton et al (8) in a systematic review, reinforced that an increased NLR is associated with a poorer outcome across many solid malignancies. Templeton et al determined a median cut-off value for the NLR of 4 and this was associated with a hazard ratio for the overall survival of 1.81 ($p<0.001$). That is, a higher NLR was associated with a worse outcome (8).

NLR has also been examined in cardiovascular disease. Bhat et al (9) showed that an increased NLR predicted an increase in both short and long term mortality following an acute coronary syndrome. Furthermore, the NLR seems to be an independent predictor of outcomes in stable coronary disease and cardiac failure (9).

Studies of NLR in pregnancy are limited to complications of pregnancy or diseases that occur during pregnancy (3-5,10,11). Ilhan et al studied the relationship between NLR and acute pancreatitis during pregnancy to determine its value as an early predictor of the disease (10). A NLR greater than 4.1 was associated with acute pancreatitis.

Kirbas et al showed a similar association between elevated NLR and cholestasis in pregnancy (3). The mean NLR in women with cholestasis was raised compared to the control group. In women with severe cholestasis the NLR was 5.59±1.98, while in a normal pregnancy the NLR was 2.32±0.77 (3).

Kurtoglu et al and Yavucan et al looked both at the association between severity of pre-eclampsia and NLR (6,11). The NLR value is significantly higher in pre-eclamptic patients compared to a non-pregnant control group (6,11). However, there was no significant difference between NLR values between healthy pregnant women and women with preeclampsia (6,11). According to the aforementioned studies, the NLR was not associated with severity of pre-eclampsia or severity of symptoms. Thus NLR appears to have no role as a screening tool or a diagnostic tool for pre-eclampsia (6,11) per se as any inflammatory condition will elevate NLR status. Canzoneri et al established that the leucocytosis observed in pregnancy is mostly due to an increase in neutrophils, with no significant change in lymphocytes. (5)

2. Data collection and study methods

The data was collected at Albury-Wodonga-Heath in New South Wales and Victoria in Australia. All women in this study were confined at Albury Wodonga Health (AWH), Wodonga Maternity Unit, which services the border towns of Albury and Wodonga. Albury Wodonga Health is a secondary obstetric centre on the border between the Australian federal states New South Wales and Victoria. Complicated pregnancies requiring confinement before 33 weeks are transferred to tertiary centres in Melbourne. Such cases are excluded from this study.
Data was retrieved retrospectively from all women confining at AWH between February 2015 and November 2015 inclusive.

Data was collected from the electronically generated patients discharge summaries or the hospital pregnancy health records. All available full blood counts which included white cell counts were collected. The goal was to collect all white cell counts taken during pregnancy. Some full blood count results were not available in the hospital records due to different operational policies by different pathology providers.

The parameters collected from the full blood counts were the absolute numbers of neutrophil granulocytes, lymphocytes, monocytes, eosinophil granulocytes and basophil granulocytes. Only one full blood count per calendar day and per mother was used in this study. If there were multiple blood counts per day, the chronologically first one was recorded.
Most women have a full blood count done on admission to the hospital when they come in to labour, this was the last recorded FBC. No FBC were recorded during the stay at the hospital.

985 women who birthed at AWH between the 1st July 2014 and 28th of February 2015 were entered in to the database. Stillbirths and all births prior to 37 weeks of gestation were excluded.

The population of this study averaged 3.17 full blood counts (FBC) per woman. Some diseases or pregnancy complications required additional full blood counts and this needs to be taken into consideration.

28 patients had no full blood count results available for data collection. They were excluded from the study. This left a total number of 958 women for analysis.
The highest number of full blood count results for a patient was 10 during the time of pregnancy. The lowest number of valid blood counts was 1.

For the sake of better readability, the absolute number of neutrophil granulocytes and lymphocytes will be recorded as a simple 3-digit number, leaving out the $*10^9$ at the end of each number.

Besides the values necessary to determine the NLR-ratio the following data was entered in to the data base: Maternal age (integer numbers); estimated due date (early ultrasound was favoured over menstrual dates. Most women had a 10-week dating scan and almost all had a 20 weeks morphology scan); parity; antenatal complications; labour complications and estimated peri-partum blood loss. Parameters of labour like number of previous children, complications during labour and blood loss were collated as well.
The pre-pregnancy weight or the earliest pregnancy weight and the maternal height were recorded and a BMI calculated. The pre-pregnancy weight was subtracted from the last available ante or intrapartum weight and the gestational weight gain was calculated.
Fundal heights are usually recorded antenatally. The most recent fundal height measurement within 14 days of delivery was entered in the database.
The following neonatal data was retrieved: The gestational age at birth, the neonatal length, head circumference and weight. The neonatal length and head circumference were rounded to whole centimetres; the neonatal weight was recorded in 10 gram intervals.
The NLR has been calculated by dividing the absolute number of neutrophil granulocytes by the absolute number of lymphocytes.

3. Results

In Figure 1 the distribution of the NLR over the days of gestation is shown. The days of gestation are drawn on the x-Axis while the NLR are drawn on the y-Axis. Every measurement is plotted individually, thus the number of data points is 3115.

It can be seen, that the medical checks focus on 3 periods. The clusters of values are due to the recommended blood counts at 10, 28 and 36 weeks of gestation. This translates to 70, 196 and 252 days of gestation, this is where the clusters are located.

It should be mentioned that Figure 1 is cropped due to very few high values of the NLR. This means that the graph does not show NLR values over 16. The maximum value for the NLR in the dataset is at 33. 36 Values were between 33 and 16. It can be assumed, that this values most likely are outliers. Therefore, and for better readability these high values are not plotted in Figure 1.

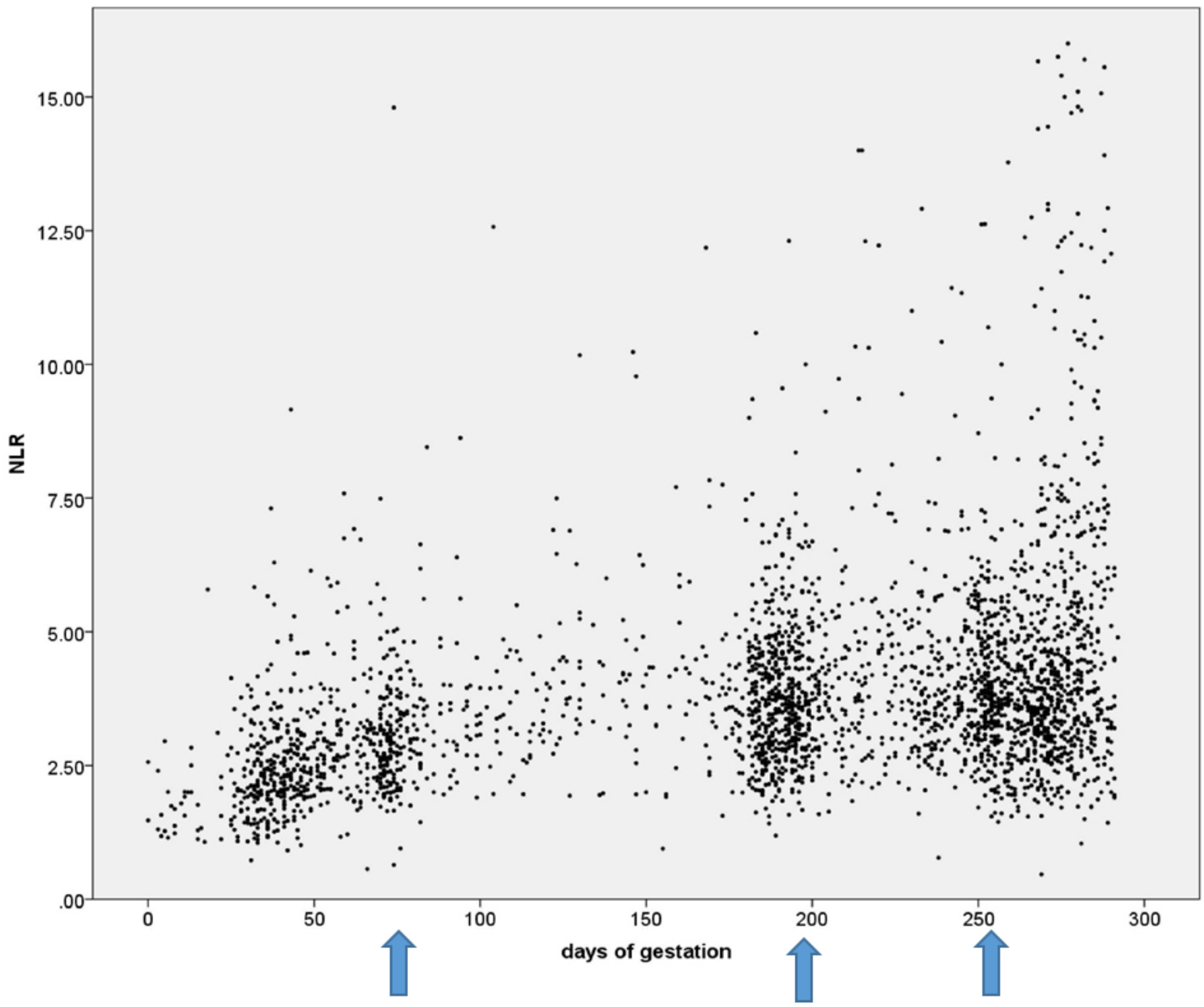

Figure 1: Scatterplot of the NLR over course of pregnancy (days of gestation). The arrows indicate the recommended time of blood count.

3.1. Age distribution of the expected mothers:

The following graph in Figure 2 shows the age distribution of all patients in this study. For all 956 patients a recorded age was available. The mean age was 29 years (SD ± 5.48) rangingfrom 14 to 44 years. The median is also at 29 years of age.

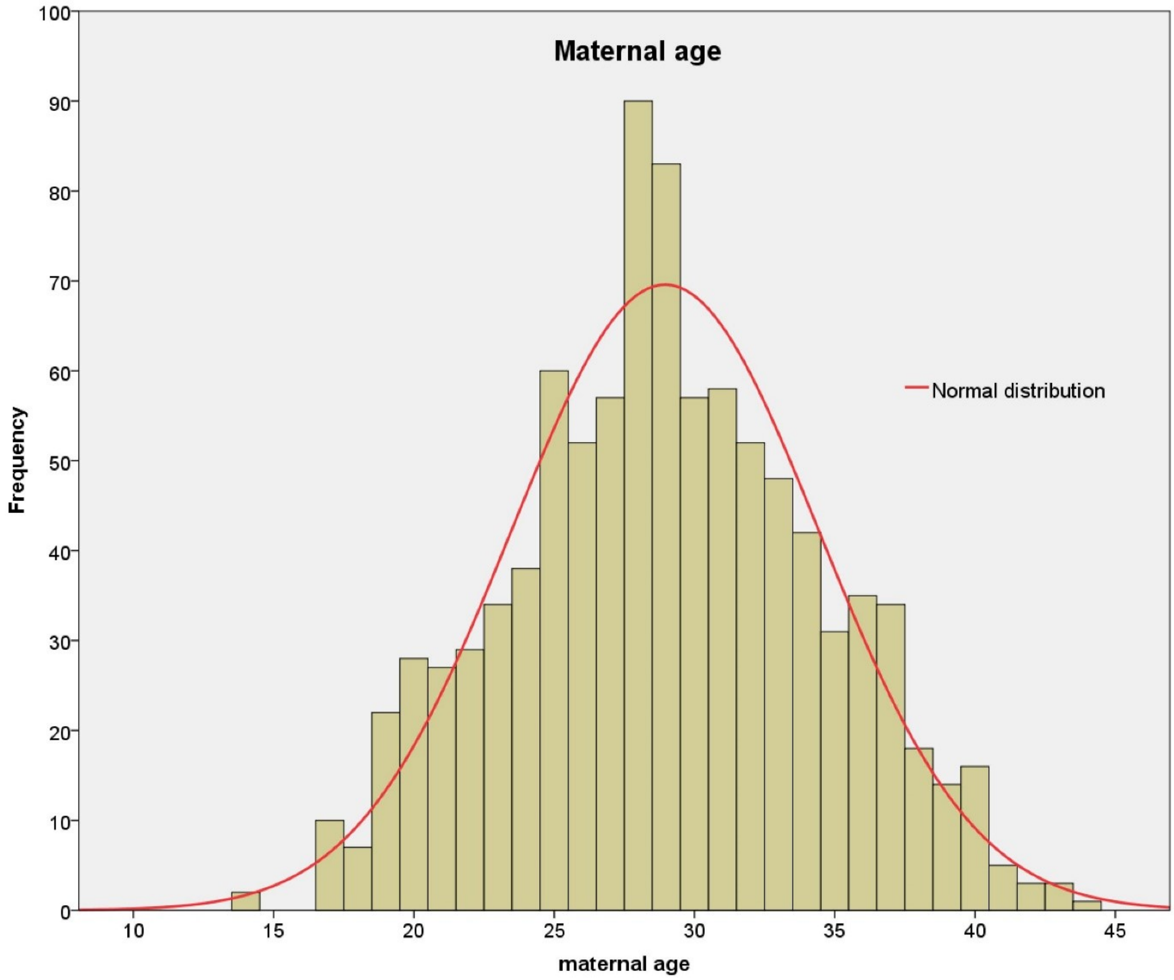

Figure 2: Age Distribution of the expected mothers

The graph shows, that the age distribution fits very well to a Gaussian distribution. The ages of 28 and 29 years have the greatest frequency in Figure 2. There is no shift of the distribution to the left or to the right which indicates the Gaussian distribution is not skewed. This is also indicated by the median value which is at 29 years, and is nearly the same value as the mean value. Generally, the age distribution of this study follows the data collected by the Australian Bureau of Statistics in 2014. The median maternal age for the overall Australian population as calculated for 2014 was 30.9. (12) In the Federal state of Victoria where the obstetric service is located the median maternal age was 31.5 years (2014). This is a considerably higher than the population in our study whose median age is 29 years of age.

3.2. **Parity**

On an average, the new born child is the second child in our population group. The mean value in the dataset is 2.01 children per woman, including the current pregnancy. The mean number of times a woman has given birth in Australia was 1.87, so the data set under investigation is slightly above this value.

Figure 3 shows the parity of the patients. This means the number of previous and the actual births per woman. It shows that for over two thirds of the women it is either the first or second child. The bar graph in Figure 3 shows an exponential decline in parity, with only a few women in the cohort having 4 or more children.

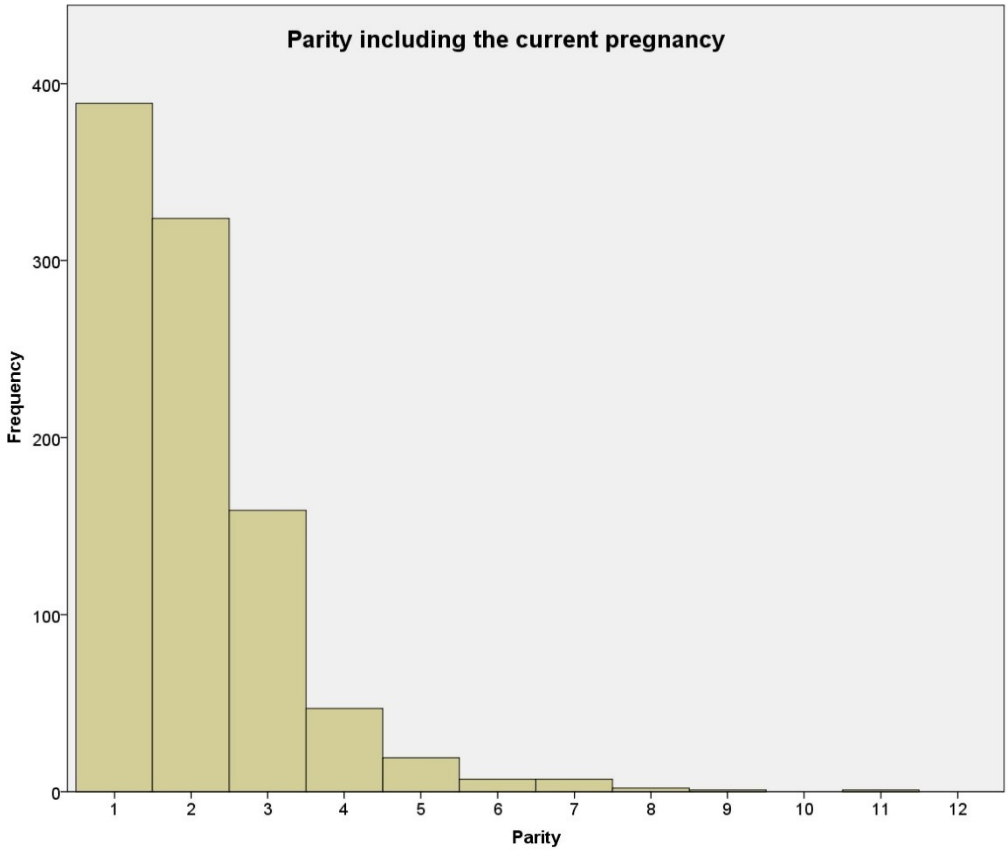

Figure 3: Parity including current pregnancy

3.3. The NLR

Within this chapter at first the available data on the neutrophil granulocytes and the lymphocytes are analysed independently and afterwards, the ratio of the two values (NLR) is discussed.

3.3.1. Neutrophil Granulocytes

For determining the development of the neutrophil granulocytes, all white cell counts (WCC) measurements taken during the first trimester were analysed. The first trimester ranges from 0 to 12 weeks of gestation which translates to 0 to 84 days of gestation. The absolute mean number of neutrophil granulocytes is 5.55±1.87 with a range of 13.5. The range is defined as the difference between the highest and the lowest value in the given data. It is very notable that in most graphs the full range is not visible since the highest value is left out due to better visibility.

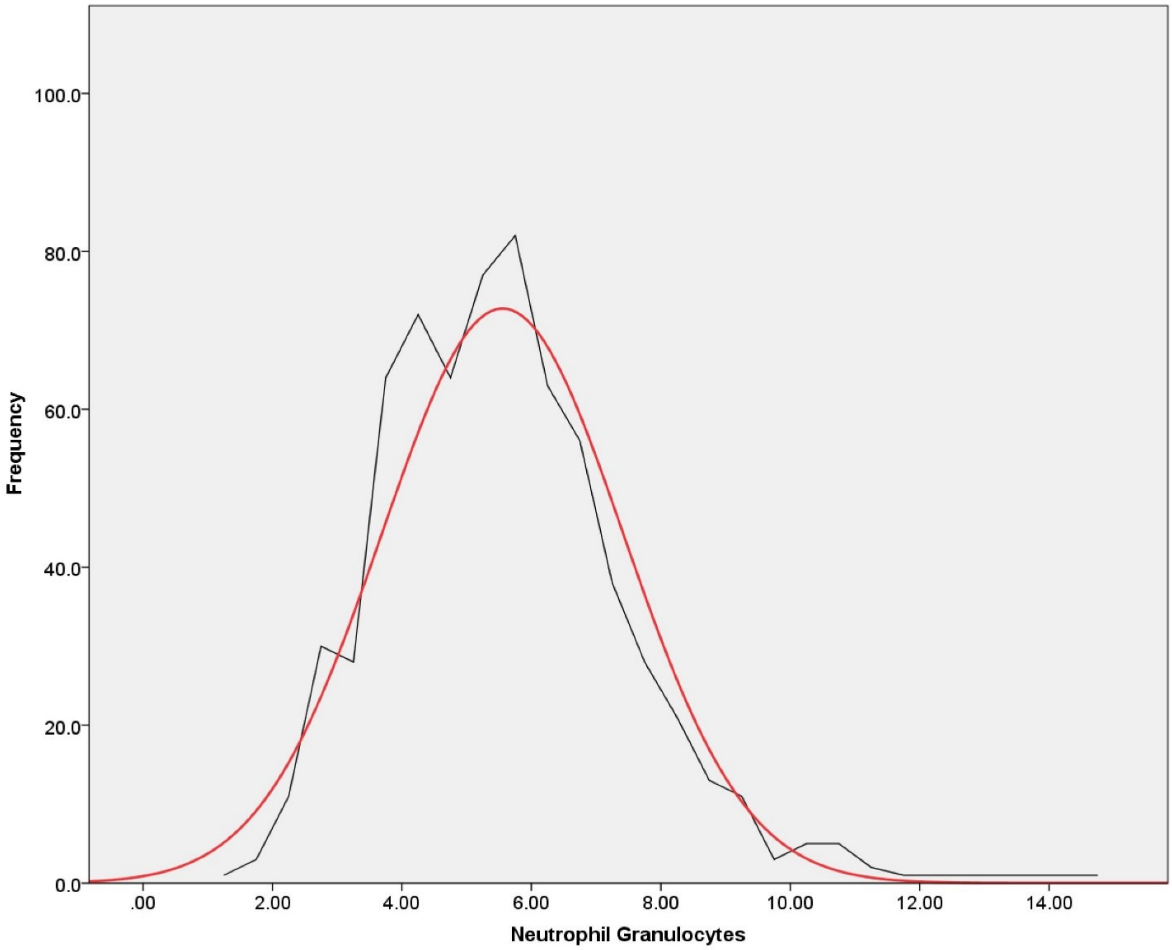

Figure 4: Histogram of the neutrophil granulocytes in the first trimester. On the x-axis is the absolute number of neutrophil granulocytes drawn and on the y-axis the frequency of the value (red shows a fitted normal distribution)

Figure 4 shows a graph of the distribution of the neutrophil numbers in the first trimester. he neutrophil granulocyte counts for the cohort during the first trimester follows a normal distribution well. The mean value for the data set under investigation is slightly below 6as above mentioned. Also the mean value of the standard distribution is at 6. This indicates, that no great skewness of the distribution is present.

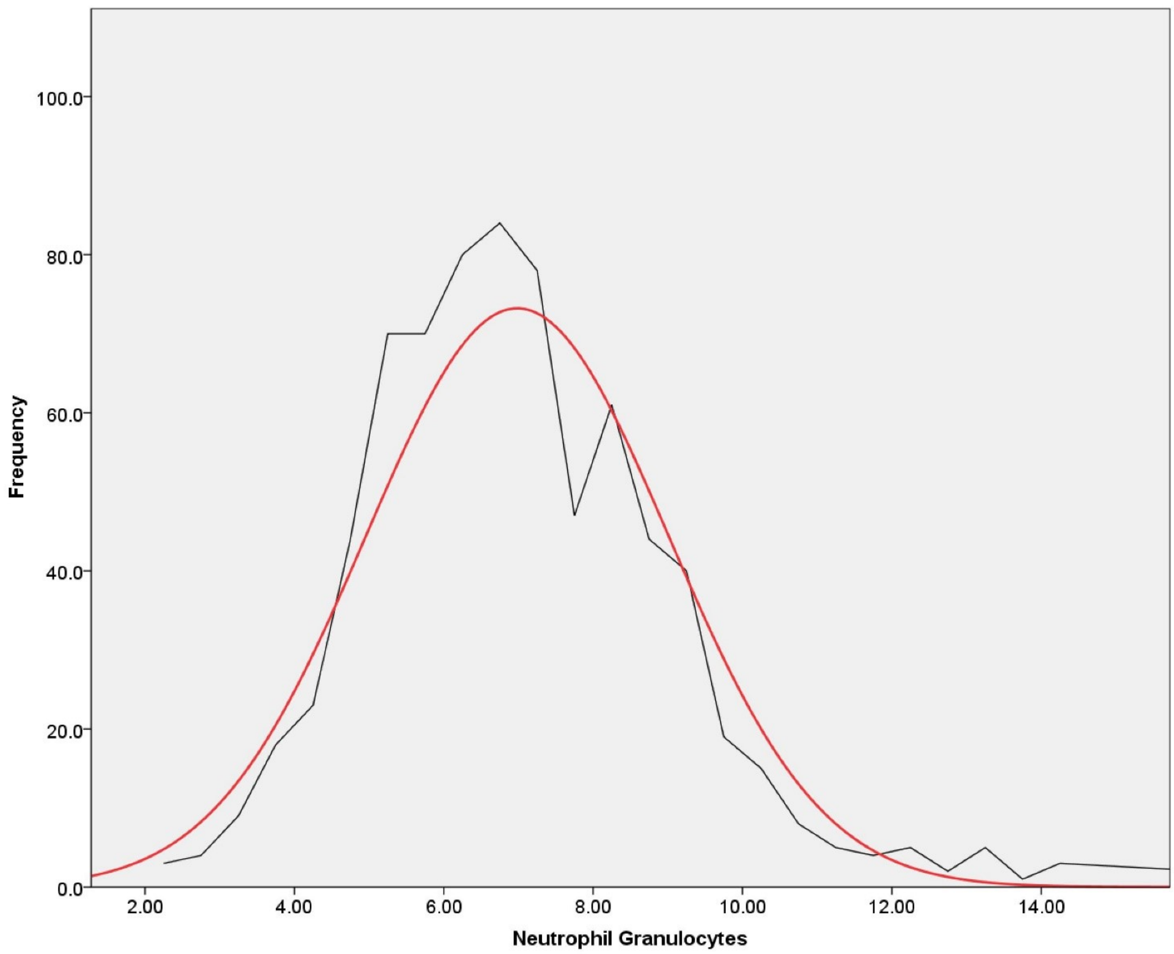

Figure 5: Histogram of neutrophil granulocytes in the second trimester

The mean number of neutrophil granulocytes increases from 5.55±1.87 in first trimester to 6.98±2.03 in second trimester. The second semester was defined to range from week 13 to week 28 thus including days 85 to 196. The range has increased slightly from 13.5 to 14.05.

Figure 5 shows a line histogram of the neutrophil granulocytes number in second trimester and depicts a normal distribution, note the mean values for the population is increasing. There is also a very notable increase in the values above 8.

The distribution of the neutrophil granulocytes fits with the standard distribution. However, compared to the first trimester, the matching is not as good. The mean value is at 6.98. The calculation of median of the data set under investigation is at 6.79.
This indicates, that a slight skewness of the distribution is present. But the fact, that among the higher values of the neutrophil granulocytes values with measurement errors are present has to be taken into account.

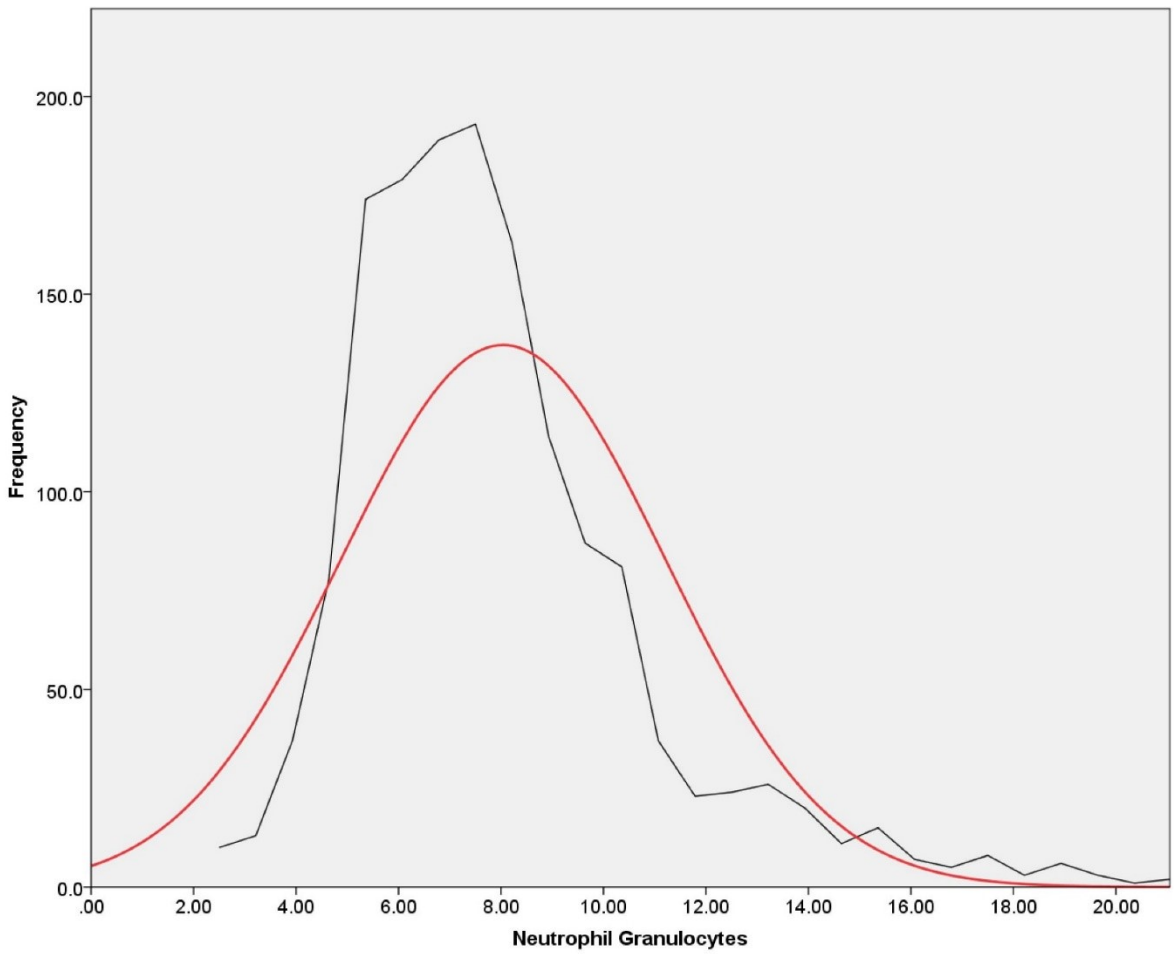

Figure 6: Histogram of the neutrophil granulocytes in the third trimester

The third trimester was defined to range from week 29 onward until delivery and thus onward from day 197.

In the third trimester the mean number of neutrophil granulocytes increases further from a mean of 6.98±2.03 to 8.04±3.16. The range is increasing even more from 13.5 in second trimester to 28.6 in third trimester. In Figure 6 the data are shown. It demonstrates an increased frequency of higher neutrophil counts. For the data of the third trimester also a normal distribution was calculated and is shown in Figure 6 (red curve). It can be seen that this distribution does not fit as good as the previous two. The curve in Figure 6 is more skewed and thus a Rayleigh distribution would be a better fit.

Comparing the results of the three trimester shows, that not only the the mean value and the range of the value are increasing with incrising trimester but also the distribution of the values changes their character significantly. This relates to the status of the pregnancy.

Figure 7 summarizes the mean number if neutrophil granulocytes per trimester including the standard deviation. The mean number of neutrophils in the first trimester is 5.55 which increases to 6.98 in second trimester and 8.04 in the third trimester.

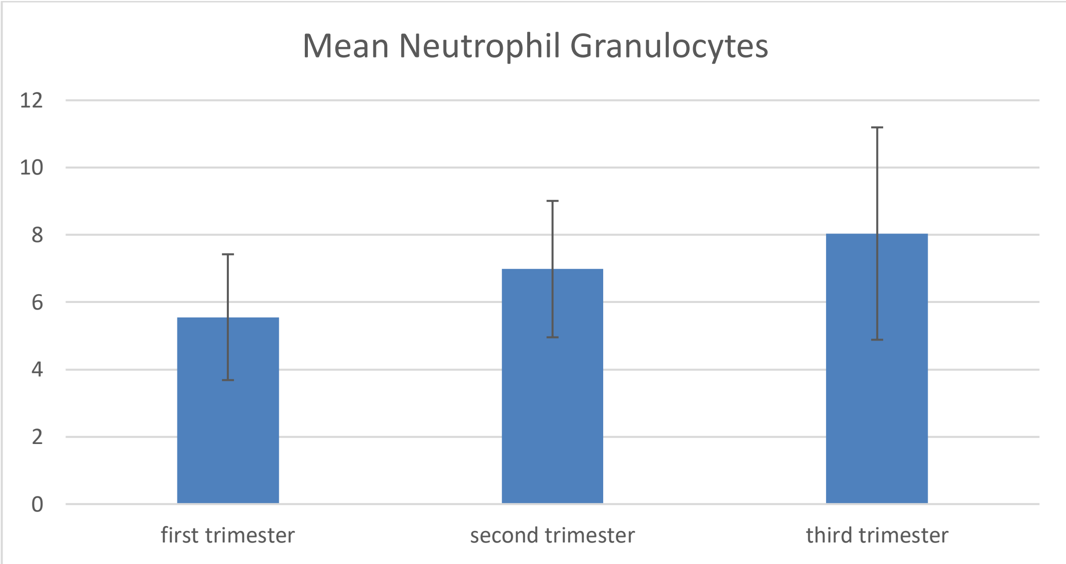

Figure 7: Mean neutrophil granulocytes values per trimester including standard deviation

The standard deviation increases also from ±2.03 in second trimester to ±3.16 in third trimester.

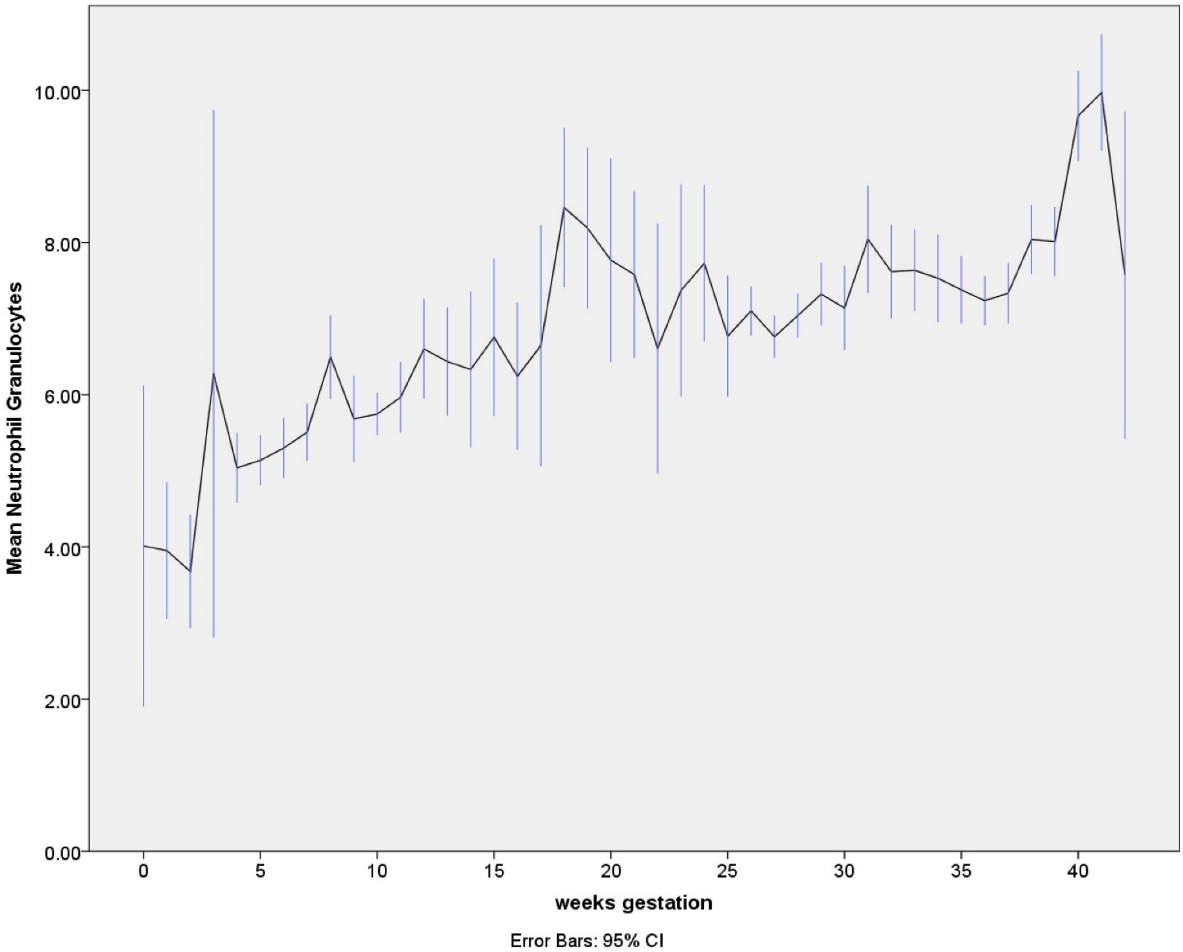

Figure 8: Mean number ob neutrophil granulocytes over weeks of gestation. The blue lines show 95% confidence intervals.

In Figure 8 the increase seems to be visible over the course of pregnancy. The graph shows the mean neutrophil granulocytes per week including confidence intervals. The increase in the absolute number of neutrophil granulocytes with advancing gestation is clearly visible. The mean absolute neutrophil number is shown on the y-axis while each week of gestation is indicated on the x-axis.

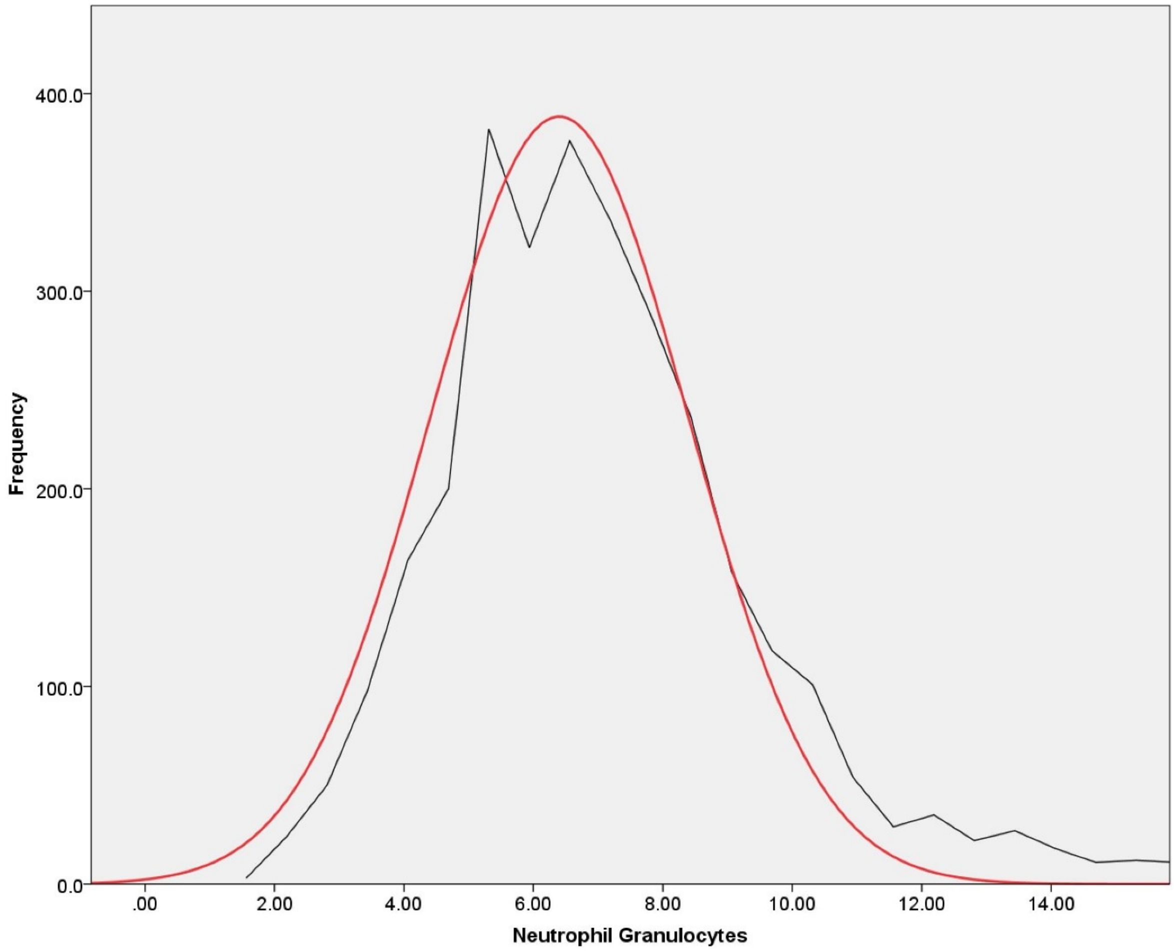

Figure 9: Histogram of neutrophil granulocytes measurements of all trimesters

Figure 9 shows the distribution of the neutrophil granulocytes for all three trimesters. This means, all data are used with no clustering in three regimes.

Again a normal distribution for this data is plotted in Figure 9. It shows, that the distribution of granulocytes is slightly skewed to the right with a noticeable amount of data points exceeding a neutrophil count above 10. For the purposes of this study these high values were treated as outliers i.e. all data point over the value of 10 were excluded in Figure 10. This means a total of 360 individual data points was excluded, this is 8,7% of the data.

Figure 10 shows the mean NLR versus the number of weeks of gestation, but this time all values over 10 have been treated as outliers and are not included in comparison to Figure 9 were all the values are included.

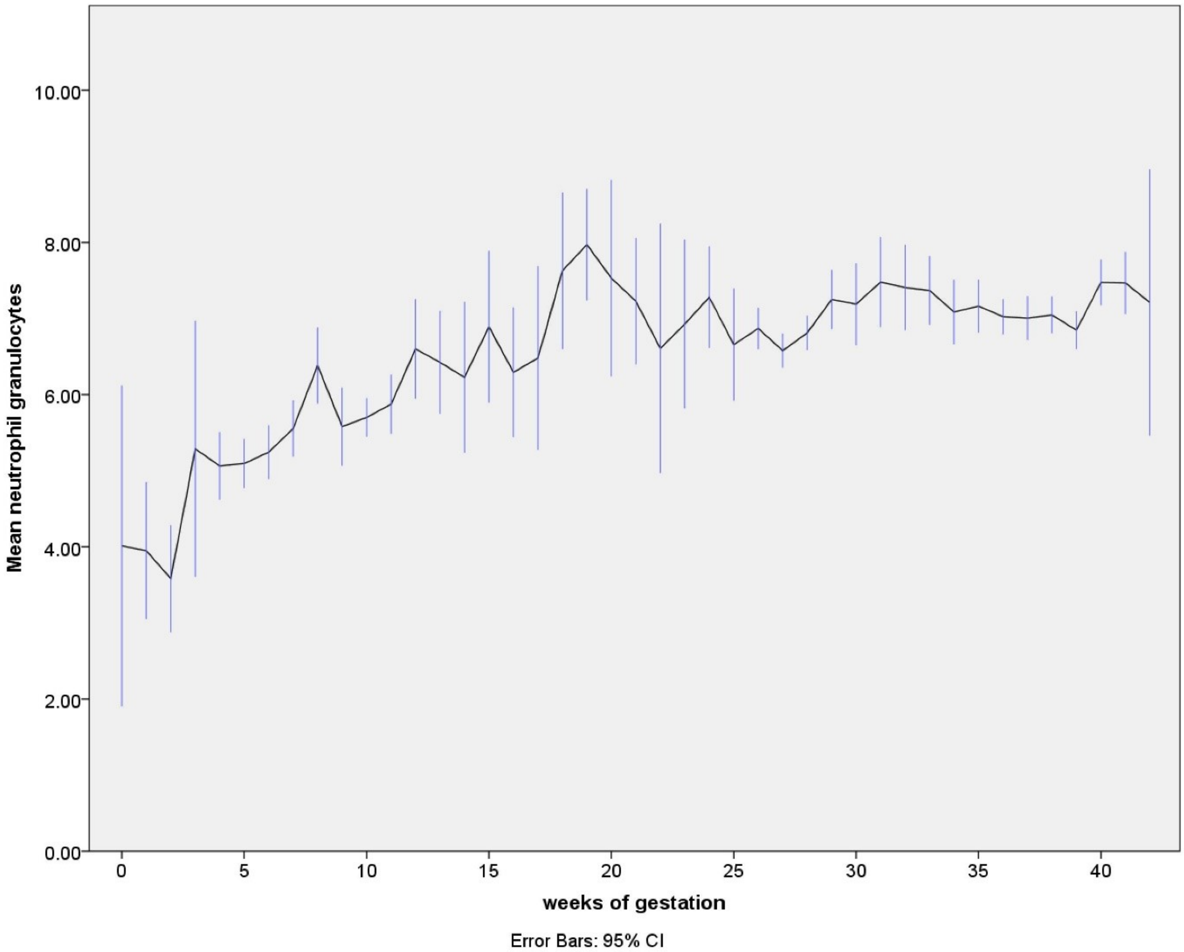

Figure 10: Neutrophil granulocytes over time excluded outliers

The result is that the 95% confidence intervals are smaller. It is also noticeable that the previously observed increase of the neutrophil granulocytes after week 35 (Figure 8) is no longer visible. This indicates either the number of outliers increases at the end of pregnancy or there is an increase in neutrophil granulocytes in certain patients at the end of pregnancy. Since it is not very likely, that the number of outliers will increase with increasing weeks of gestation, it is very likely, that the neutrophil granulocytes increase at the end of the pregnancy.

3.3.2. Lymphocytes

As with the neutrophil granulocytes the lymphocytes were analysed per trimester.
Figure 11 shows a line histogram of the lymphocytes in the first trimester. The mean value for the lymphocyte count is 2.11±0.59.

The normal distribution (red) in shows a good adherence to the plotted data curve, with the exception of two spikes around the maximum frequencies. Using the same approach as above it could be stated that in contrast to the neutrophil granulocytes the number of outliers is small since the normal distribution fits very well. Even with considering the two spikes the curve is barely skewed in the regime of the first trimester.

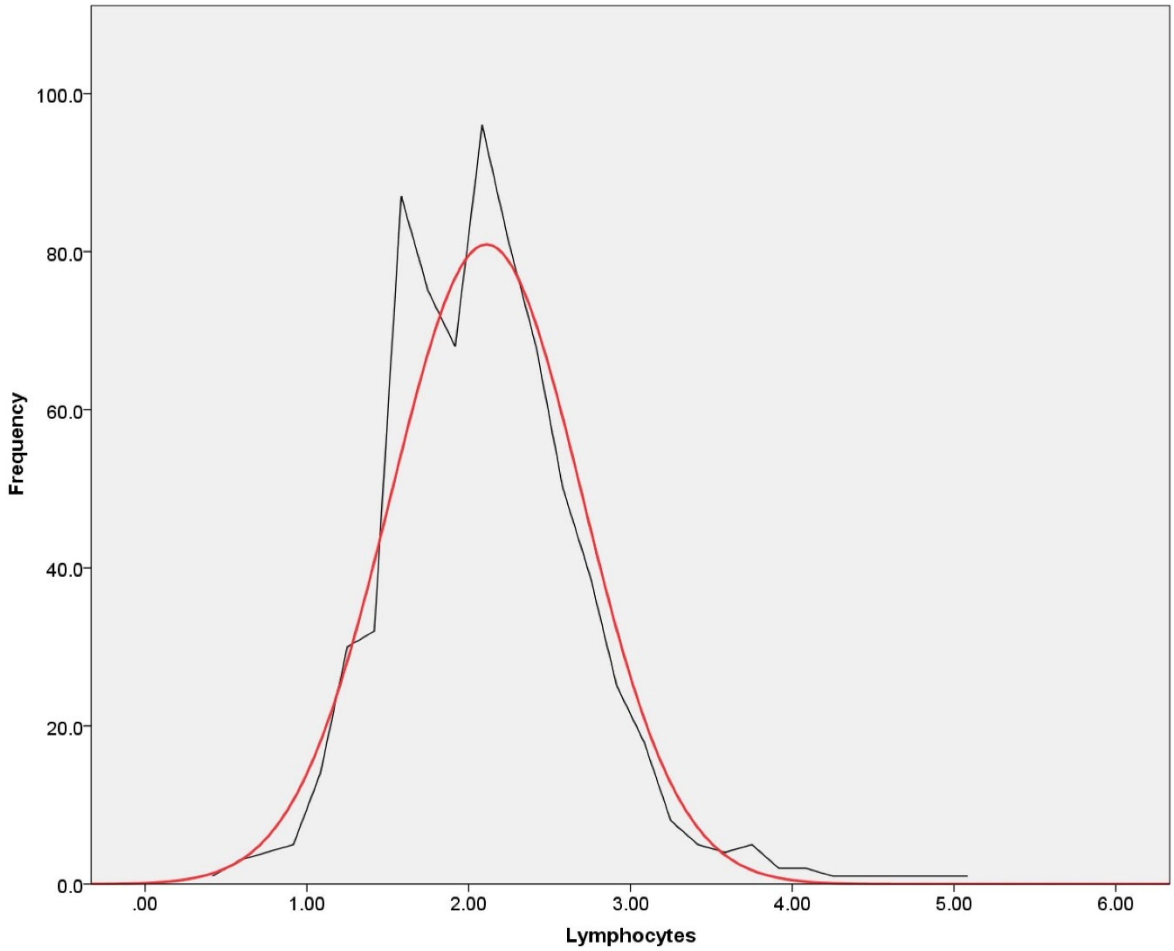

Figure 11: **Histogram of lymphocytes in first trimester**

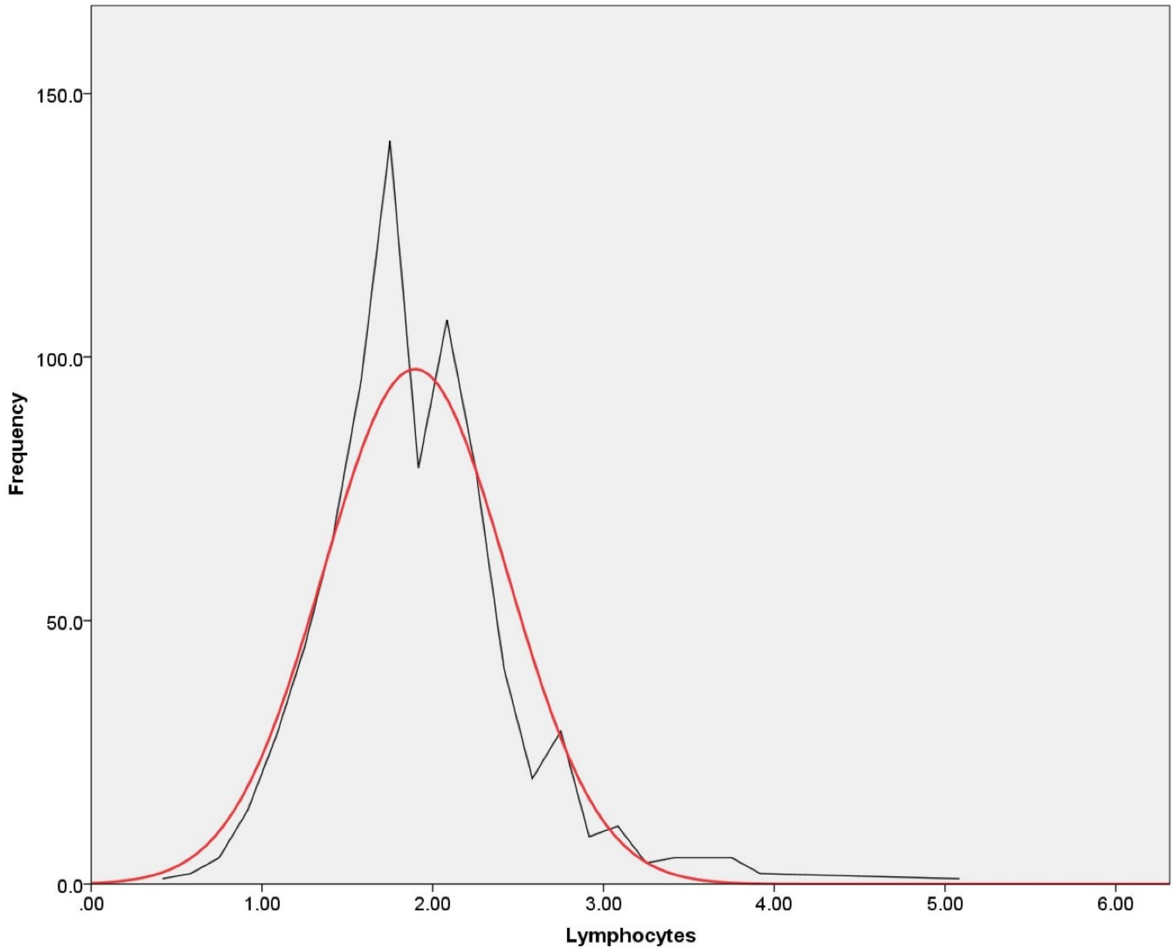

Figure 12: Histogram of lymphocytes in second trimester. The distribution of values follows a normal distribution fairly well, but there is one distinctive peak at about 1.8.

The distribution of the lymphocytes in the second trimester is shown in Figure 12. For the mean value there is a small decrease in the absolute number of lymphocytes from 2.11±0.59 to 1.90±0.54. The range of values is almost identical, from 4.70 in first trimester to 4.75 in second trimester. The small decrease could either be due to statistical error or the general hemodilution that occurs in pregnancy. There are also two spikes recognizable. However, they are now in the vicinity of the maximum of the normal distribution. Again, the distribution ist not much skewed and it can be observed, that the whole distribution ist shifted a little bit to the left in comparison with the first trimester.

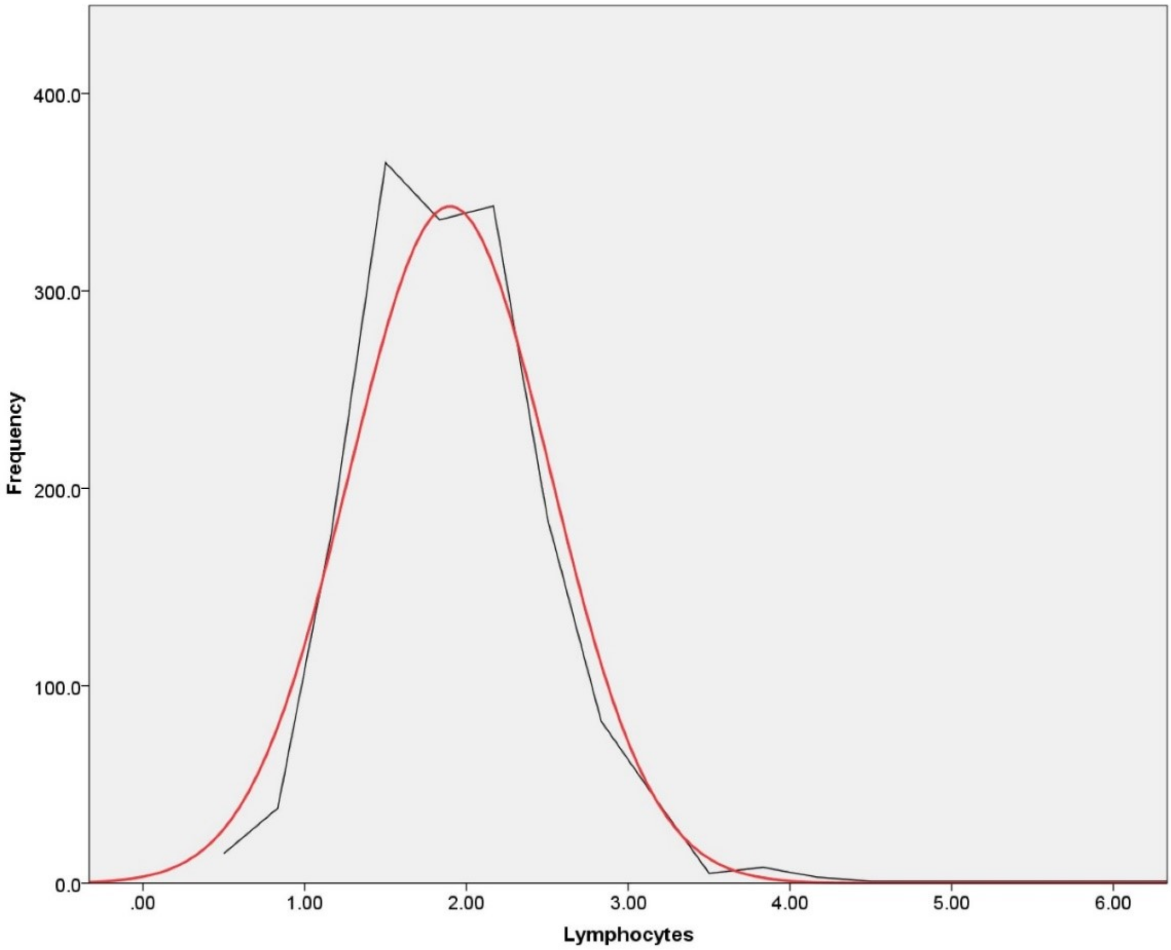

Figure 13: Histogram lymphocytes in third trimester

Figure 13 shows the distribution of lymphocytes in the third trimester. The mean value of lymphocytes stays the same at 1.90±0.62. The ranges shows an increase from 4.75 to 11.20 this is due to one very high lymphocyte count. This value can be treated as an outliers and have therefore been excluded from the data set. For the sake of better readability, they were also not shown in Figure 13 But the overall number of outliers was very small so there is no need for further analysis.

The graph as plotted shows an excellent conformity to a normal distribution (shown in red). The character of the two spikes changed also significantly. They are nearly vanished.

It is also notable, that in the case of the lymphocytes a minimal change in the standard deviation during pregnancy can be observed; 0.59 in the first trimester, 0.54 in the second and 0.62 in the last trimester. This shows that in general there has been very little change in the distribution of the values over time.

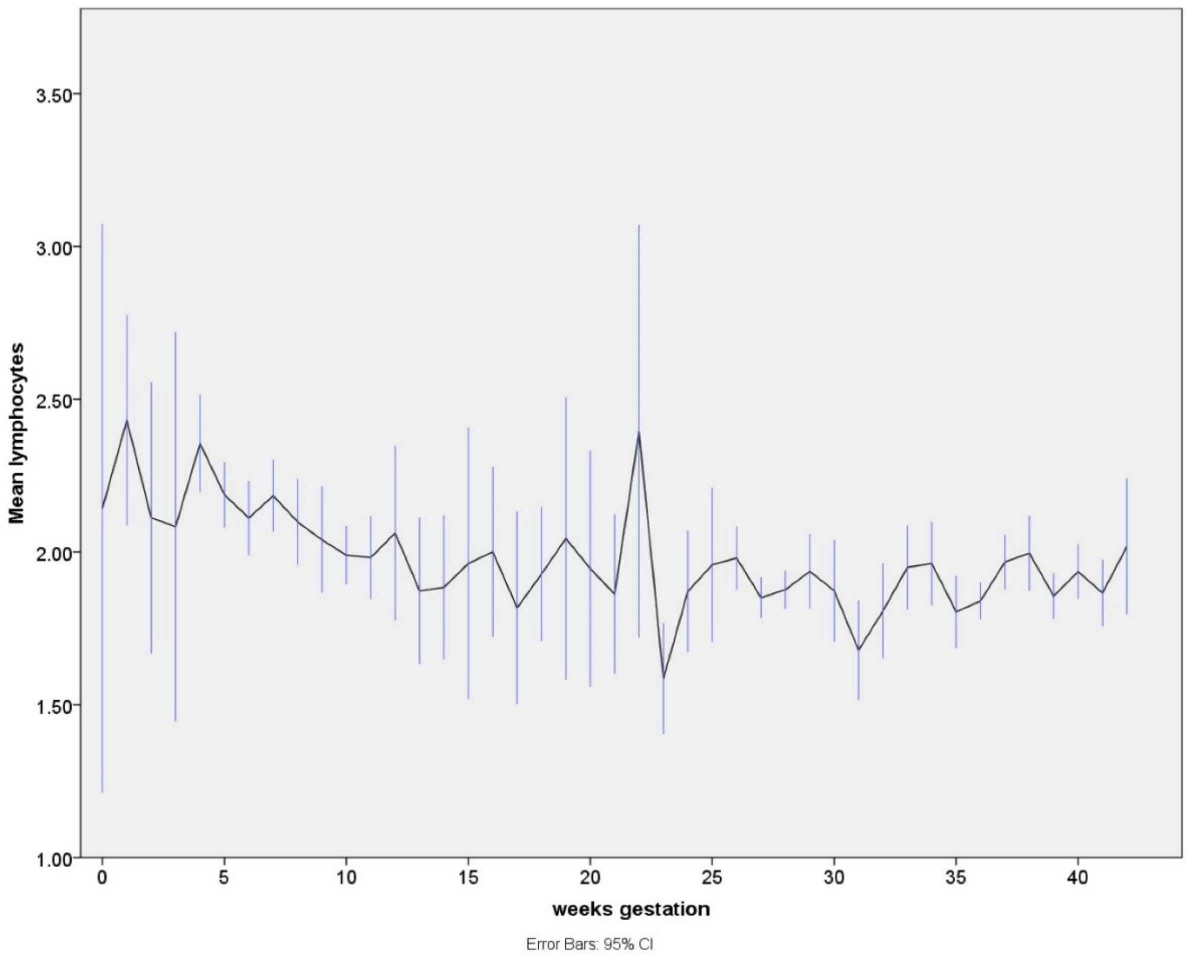

Figure 14: Mean lymphocytes over the weeks of gestation

Figure 14 shows the mean lymphocytes over the weeks of gestation. Also a 95% confidentiality intervals are shown. It summarises the results of the aforementioned graphs well, since it shows an insignificant decline in the total number of lymphocytes during the course of the pregnancy. This is most likely due to hemodilution during the pregnancy. No data points required exclusion since the range of skews was minimal.

3.3.3. The NLR

The following chapter studies the ratio of the before discussed neutrophil granulocyte values and lymphocyte values. The development of the NLR during the course of pregnancy is shown and discussed in detail in the following chapter.

3.3.3.1 NLR distribution across the pregnancy cohort

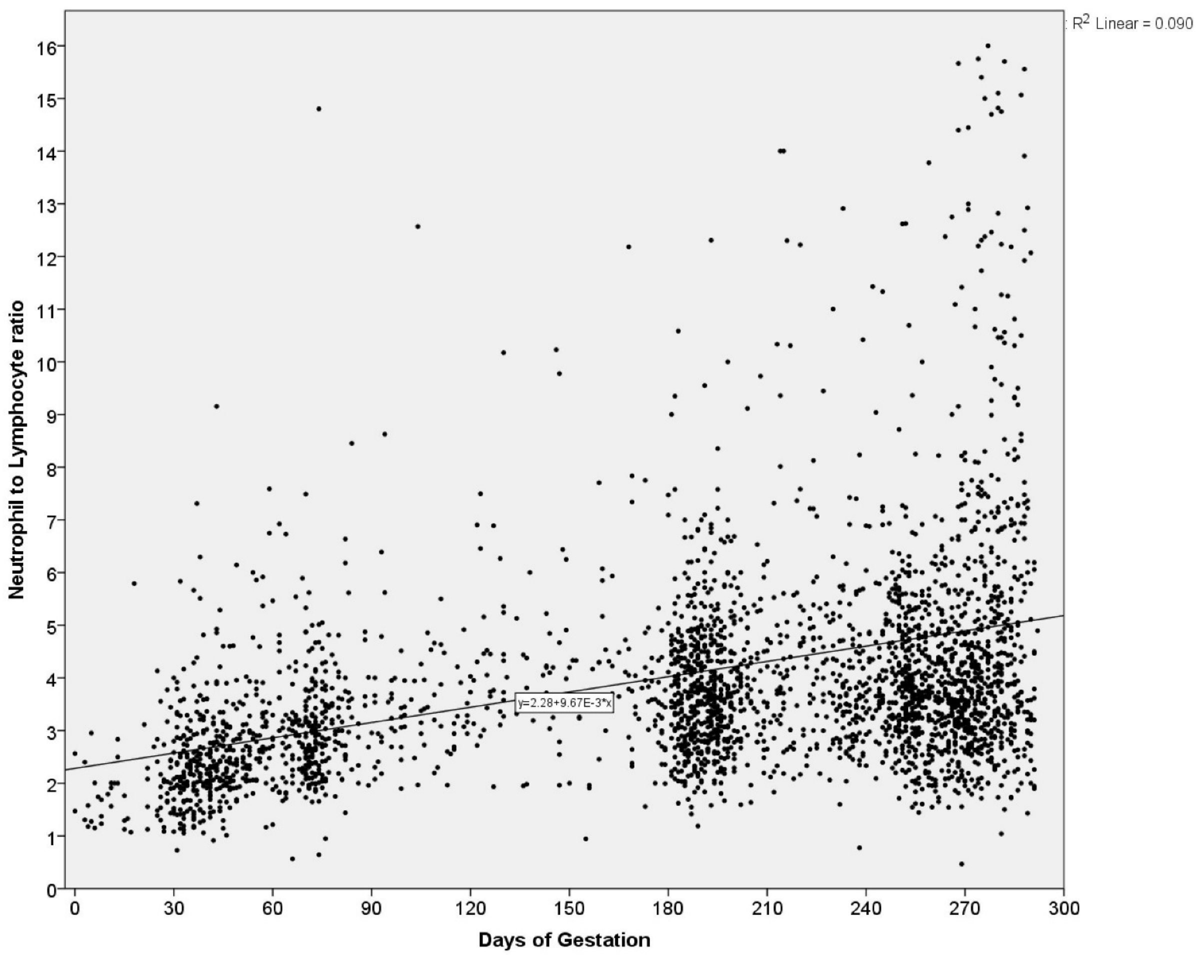

Figure 15: The NLR over the days of gestation

Figure 15 depicts a scatter plot for NLR values throughout the available time points for this pregnancy cohort.

The graph illustrates three clusters of NLR values, which represent the three recommended screening dates of 10, 28 and 36 weeks of gestation. Ten weeks equates to 70 days, which is when the ‘booking’ blood tests are done. This is in this area the recommended time. As illustrated in Figure 15, a majority of the first screening is already as early as after 30 days.

The second target date is about 28 weeks (196 days) when a glucose tolerance test is performed on all pregnant women. The data points in Figure 15 cluster very well around this date. The third target date for a full blood count including the WCC is the 36 week of gestation (252 days). This date which is close to term shows an increase in the WCC compared to previous time points. Also, there are a lot more raised values compared to the other medical scrutinies.

As there are only 36 NLR values above 16, these have been eliminated for the purposes of improving the readability of the following graphs, see Figure 15.

A Pearson's correlation coefficient has been calculated and included in Figure 15. A Pearson's coefficient is a measurement of the adherence of data points to a straight line. The Pearson's coefficient is usually expressed with the letter r whereas a r of -1 shows perfect adherence to a falling line,

$r = 0$ shows no adherence to a line whatsoever and $r = 1$ shows perfect adherence to an ascending line.

The Pearson's coefficient for our population sample was significant ($p < 0.001$), with an $r=0.3$ and an R^2 of 0.09 as shown in Figure 15. R^2 is simply the Pearson's coefficient of r squared, this is sometimes used instead of the normal r coefficient. The Pearson coefficient of $r=0,3$ indicates, that there is a great deviation from a straight line, but that still such a trend exists.

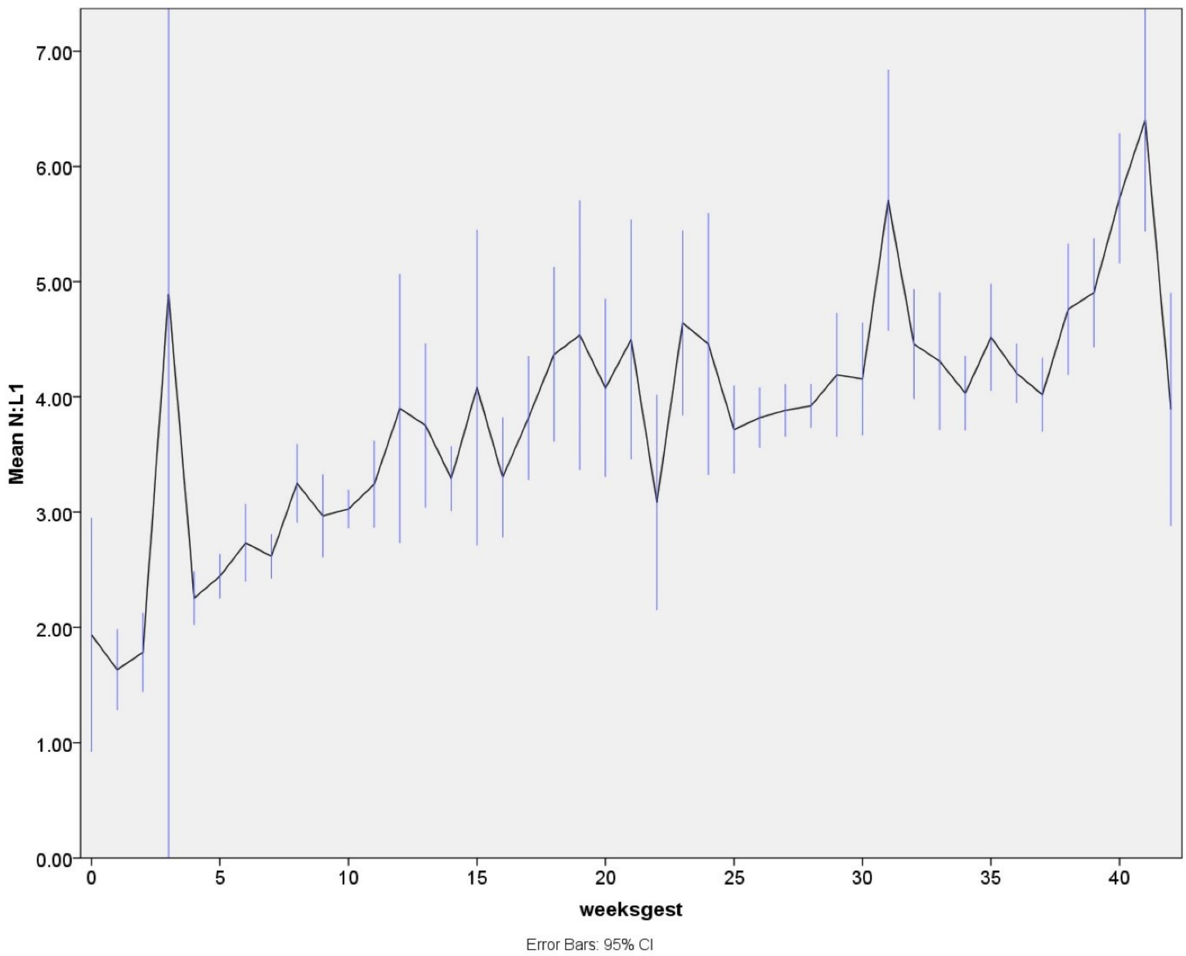

Figure 16: Mean NLR over weeks of gestation

Figure 16 shows the mean NLR versus the weeks of gestation. This graph shows, that there is a notable increase in the NLR during the course of the pregnancy.

The increasing NLR with gestation time is mostly due to the increase in neutrophil granulocytes, see Chapter 3.3.1. As discussed in Chapter 3.3.2 the number of lymphocytes decreases only slightly with gestation so that the above mentioned trend comes mainly from the neutrophil granulocytes.

For each week of gestation, a mean NLR ratio was calculated including a 95% confidence interval. However, the vertical bars, indicating the confidence level of 95%, are especially high at certain values. This indicates that there are either only very few values available or there are outliers which influence the result. Of course as a combination of both is also possible.

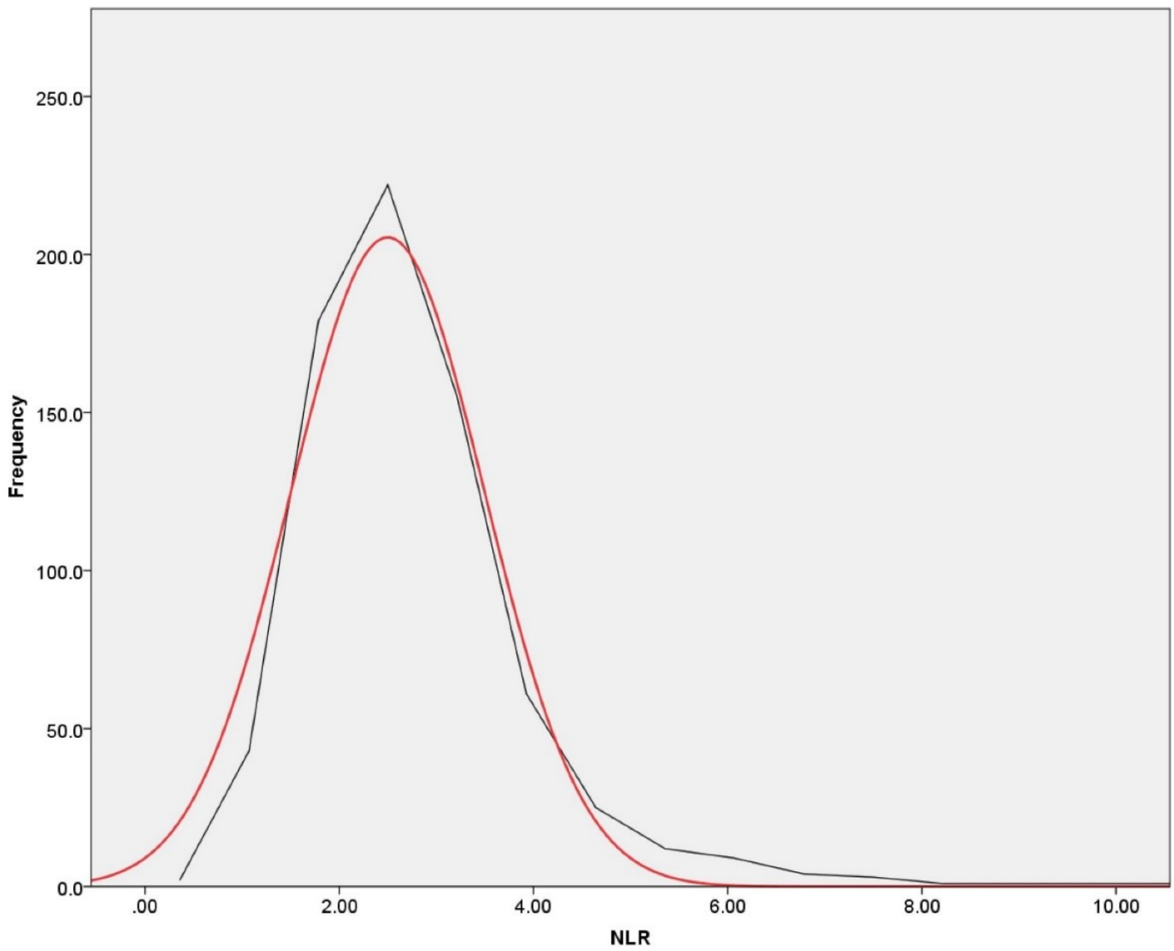

Figure 17: Histogram NLR over first trimester and shows the frequency over the y-axis and the NLR value over the x-axis.

As in previous chapters the data are grouped and discussed in three trimesters.
Figure 17 shows the distribution of NLR values in first trimester. The mean is 2.83±1.57. The absolute difference in range is high at 20.5.
The distribution shows very good adherence to a fitted normal distribution. Only a slight skewness of the curve can be detected.

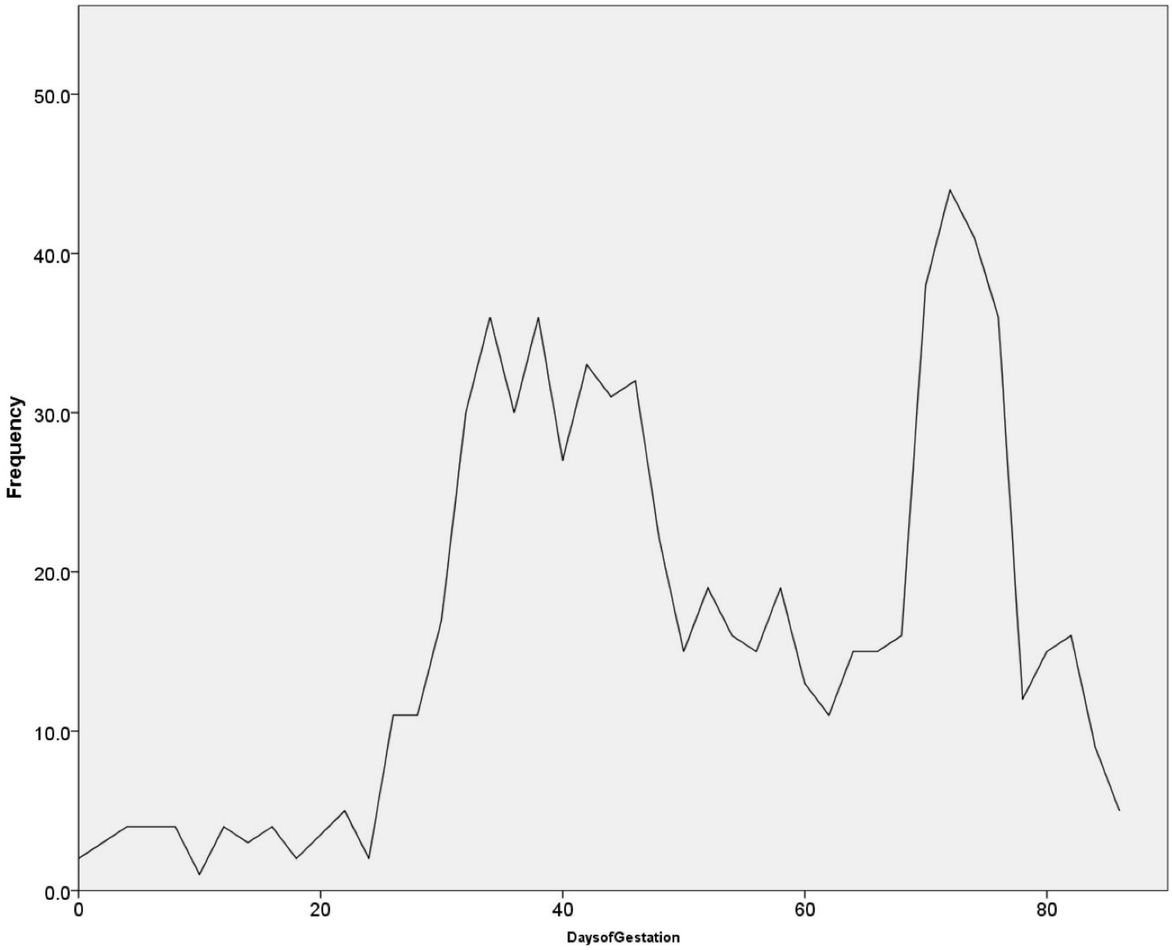

Figure 18: Histogram days of gestation and number of blood tests in first trimester

To indicate the frequency and the number of blood tests during the first trimester Figure 18 illustrates the days of gestation and the number of blood tests taken on these days (frequency). It is notable that before three weeks of gestation (21 days) the number of blood tests is very low. This can be explained by the fact that women are not yet aware of their pregnancy and have not yet consulted their medical practitioner. After 21 days the number of tests increases, most likely because a full blood count (FBC) is ordered along with a beta-HCG. The recommended date for the first screening blood test is at 10 weeks (70 days). This is depicted as the second peak in the graph.

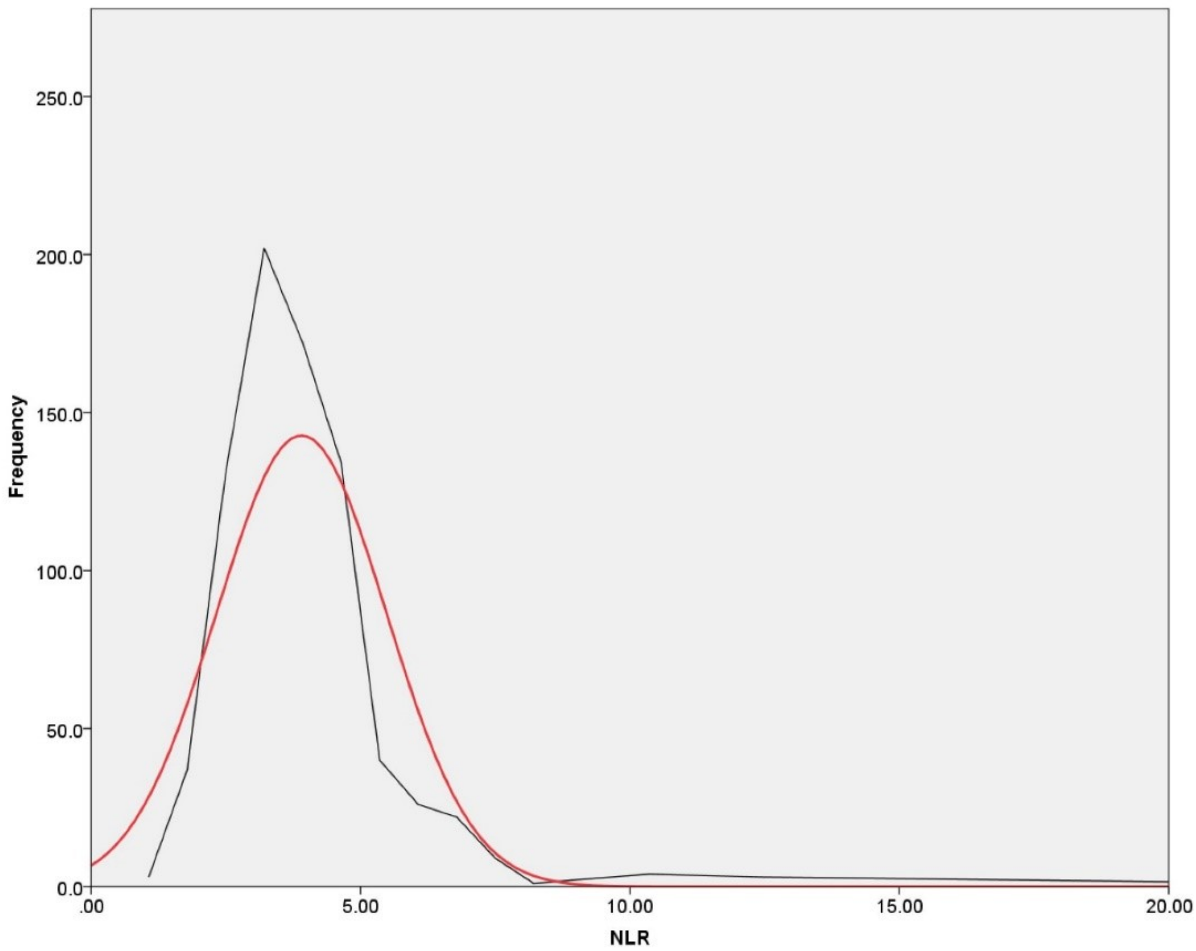

Figure 19: Histogram NLR during second trimester

Figure 19 shows a histogram of the NLR values in the second trimester. During the aforementioned second trimester, the NLR increases from a mean of 2.83±1.57 to 3.90±1.58. The standard deviation stays virtually the same. But the data set shows a less satisfactory adherence to a normal distribution curve. The range also shows virtually no change from 20 in first trimester to 21.25 in second trimester.

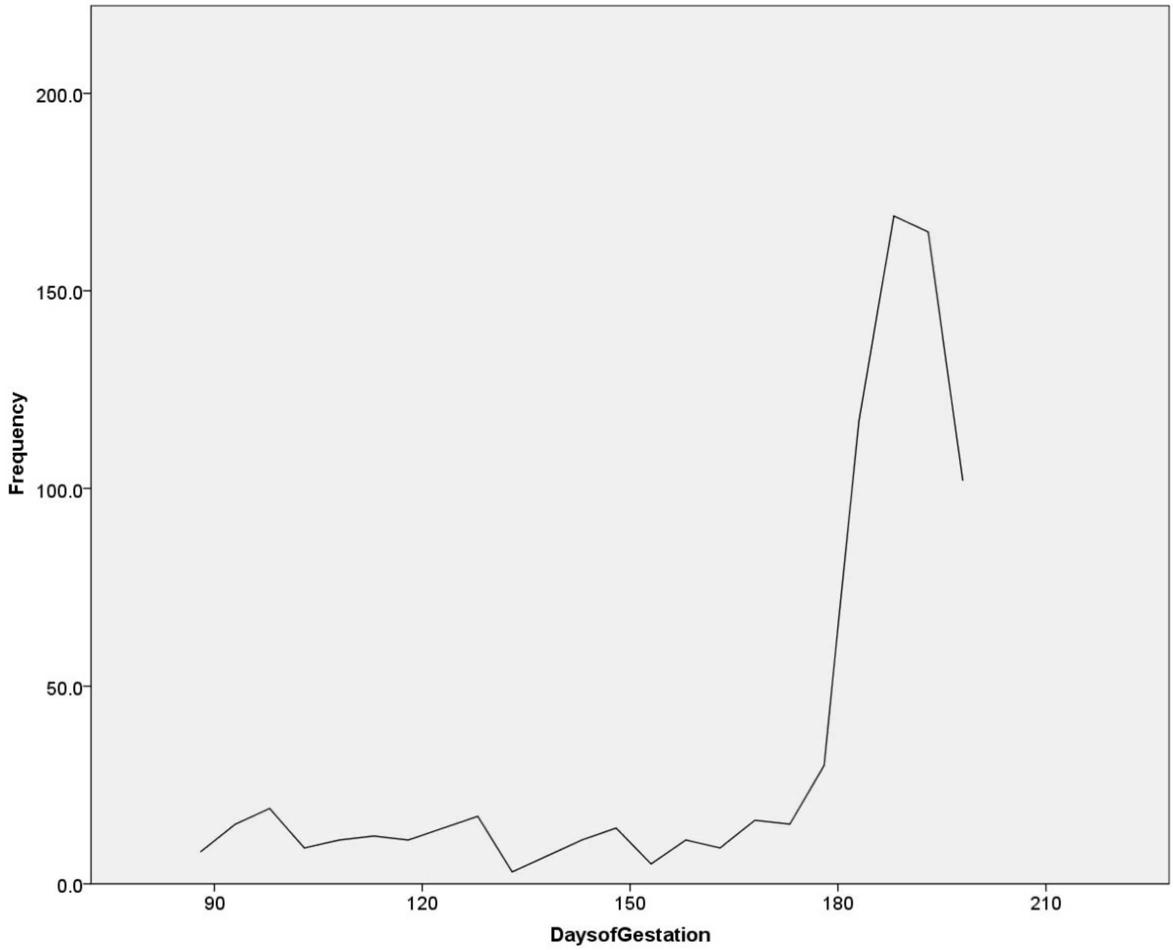

Figure 20: Histogram days of gestation in second trimester. The x-axis depicts the days of gestation and the y-axis depicts the total number of FBC's performed on that day.

Again, Figure 20 depicts a line histogram showing the distribution of the number of FBC's performed in the second trimester. The number of FBC's collected increases at 180 days of gestation. This reflects the fact that a GTT and FBC is recommended at about 28 weeks (196 days) of gestation. This explains the uneven distribution of FBC's through the second trimester and as a result the mean and median values are skewed.

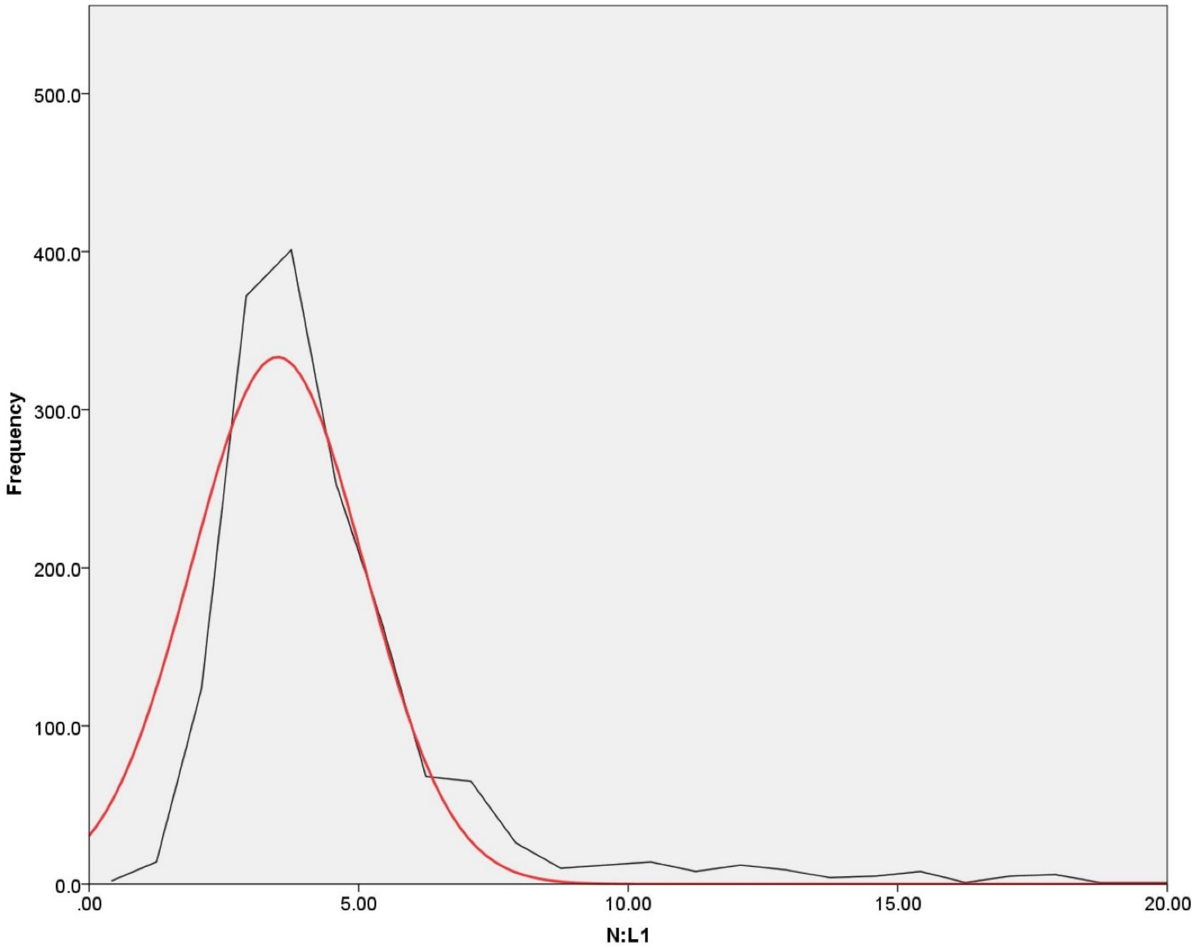

Figure 21: Histogram NLR in third trimester

In the third trimester the NLR shows a further increase from 3.91±1.58 in the second trimester to 4.77±3.37 in third trimester as shown in Figure 21 . It is notable that the standard deviation as well as the range show a steep increase. While the standard deviation increases from 1.58 in second trimester to 3.37 in third trimester, the range increases from 21.25 in second trimester to 32.53 in third trimester. It is also very notable that the number of very high NLR measurements (over NLR of 7) has drastically increased in third trimester.

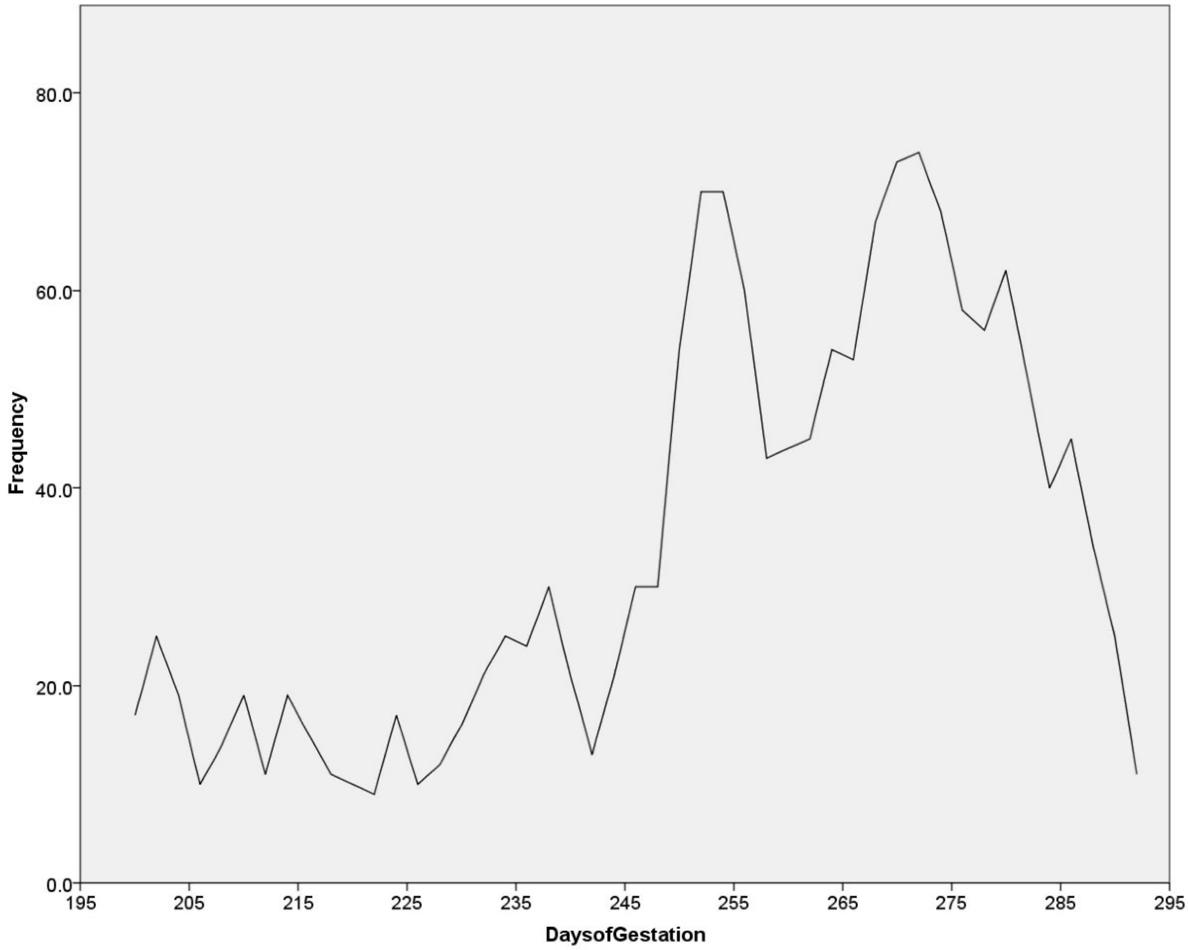

Figure 22: Histogram days of gestation in third trimester. The y-Axis shows the frequency of the FBC's taken at each specific point in pregnancy. The x-Axis shows the days of gestation.

Figure 22 is a line histogram of the point in time where the blood counts were done in third trimester. The peak starting at 34 weeks of gestation (238 days) reflects the recommended 36 weeks (252 days) FBC and the blood tests done on the day of admission. The peak is not so pronounced as for the second trimester, since at the end of the pregnancy quite a lot of individual factors are present especially the different weeks of gestations when the mother give birth to their child.

3.3.4. Dependency of the NLR ratio on other factors

There are a wide number of factors that may influence either the number of neutrophil granulocytes or the number of lymphocytes in the blood. This study has established an increase in NLR with advancing gestation in a rural maternal cohort in Australia.

In the following chapter the dependency of NLR on other factors is graphically explored. It should be studied if there are other parameters present which have an influence on the NLR-value. These include maternal age, maternal BMI and neonatal birthweight, length and head circumference.

3.3.4.1 Dependency of the NLR on neonatal parameters

It has been proposed (13) that systemic inflammation could influence the growth of the baby. The following graphs show the relationship between neonatal parameters and the mean NLR value of the mother.

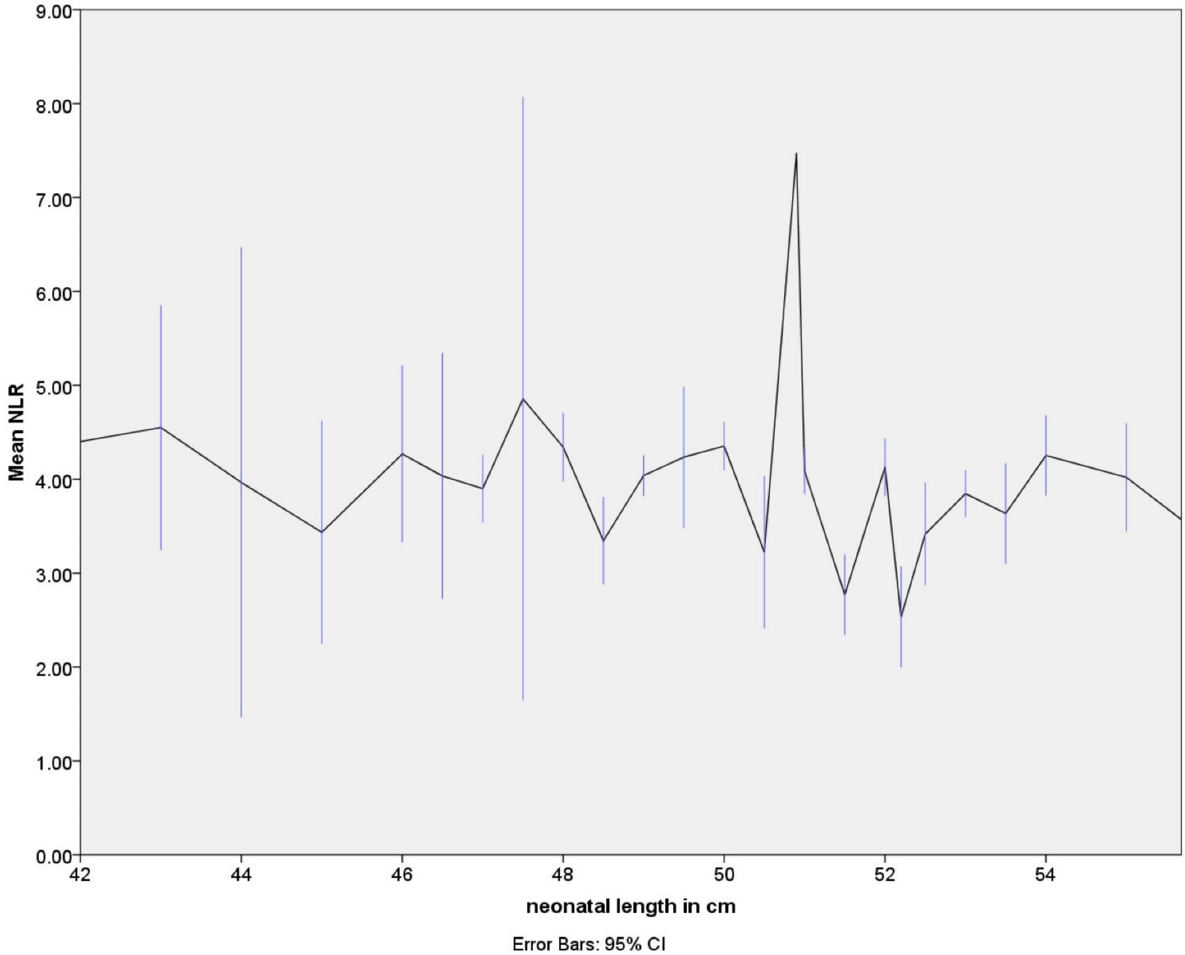

Figure 23: Mean NLR over neonatal length relationship between the NLR as drawn on the y-axis and the neonatal length at birth.

Figure 23 demonstrates that there is no significant relationship between NLR and neonatal length at birth present. This is particularly important since there are conditions influencing foetal growth such as preeclampsia and gestational diabetes for which some evidence exists for an inflammatory start, that would be reflected in an elevated NLR (6,11,14).

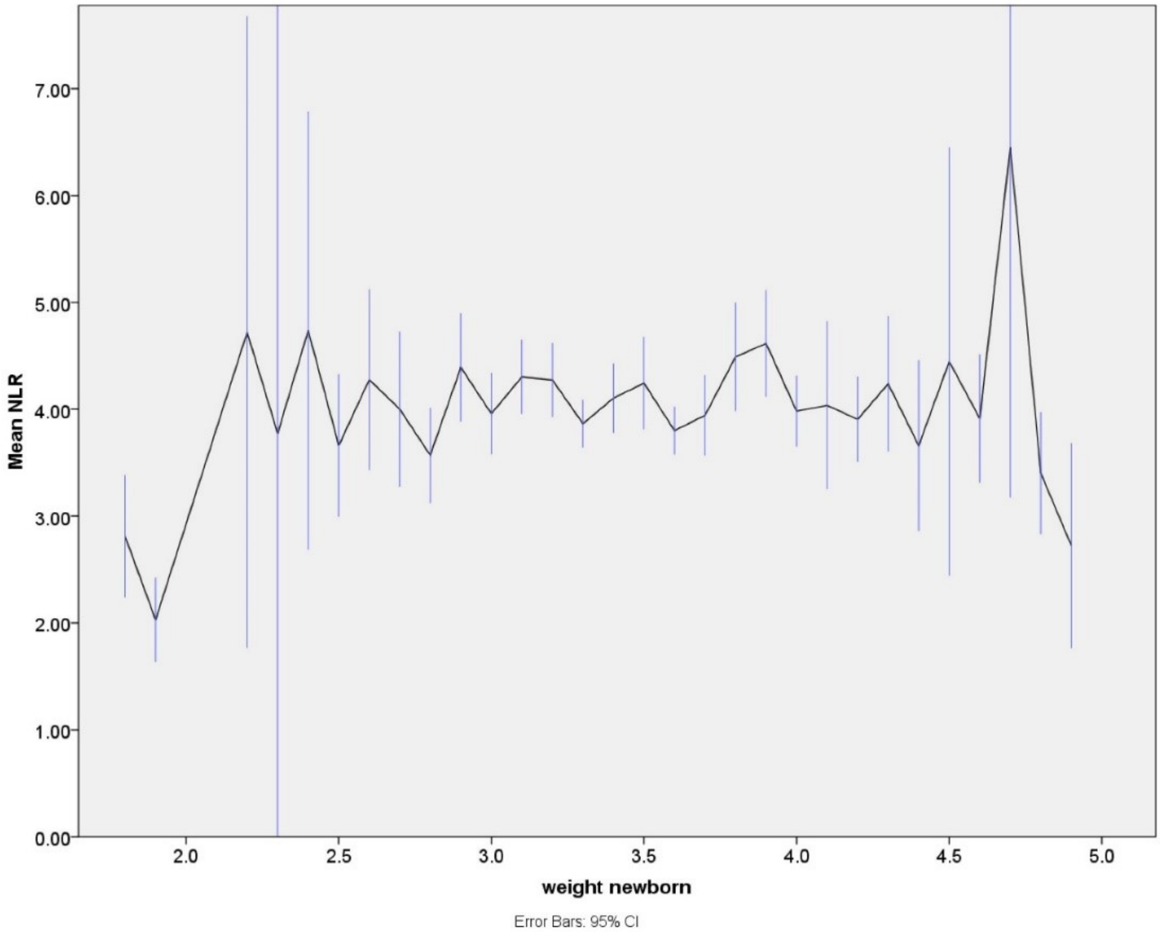

Figure 24: **Mean NLR vs. weight of the newborn**

Figure 24 shows the relationship between the NLR and an increasing foetal weight. As in Figure 23 no direct relationship is observable.

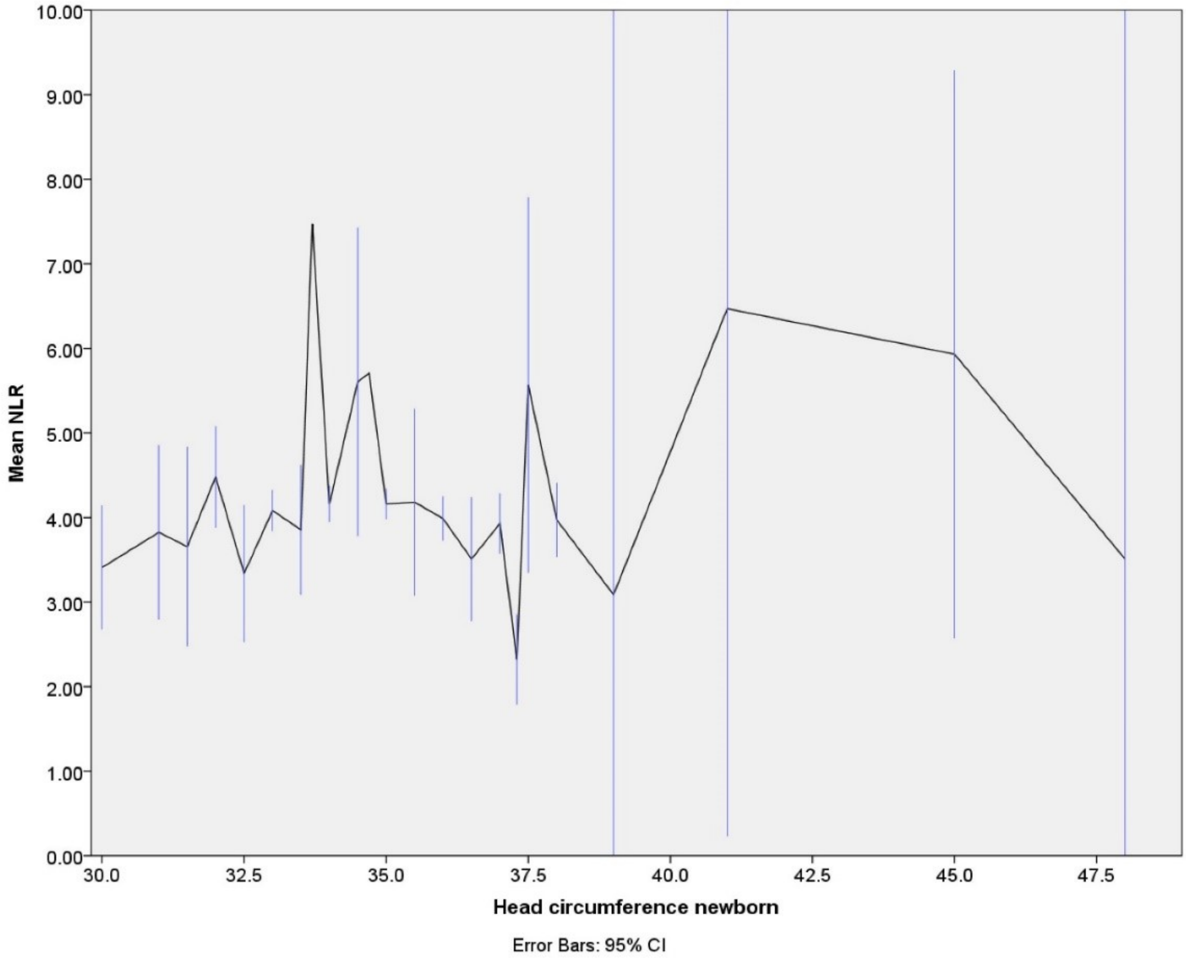

Figure 25: Mean NLR vs. head circumference. With higher head circumference measurements the number of data points grows sparse leading to wide confidence intervals.

Figure 25 shows the relationship between foetal head circumference and maternal NLR. Although the curve is ragged, there appears to be no relationship between NLR and neonatal head circumference.

In summary, there is no relationship between NLR and neonatal length, neonatal weight or neonatal head circumference. This is surprising since there are several conditions, such as gestational hypertension, preeclampsia and gestational diabetes that can influence inflammatory responses as suggested above.

3.3.4.2 Dependency in relation to the mother

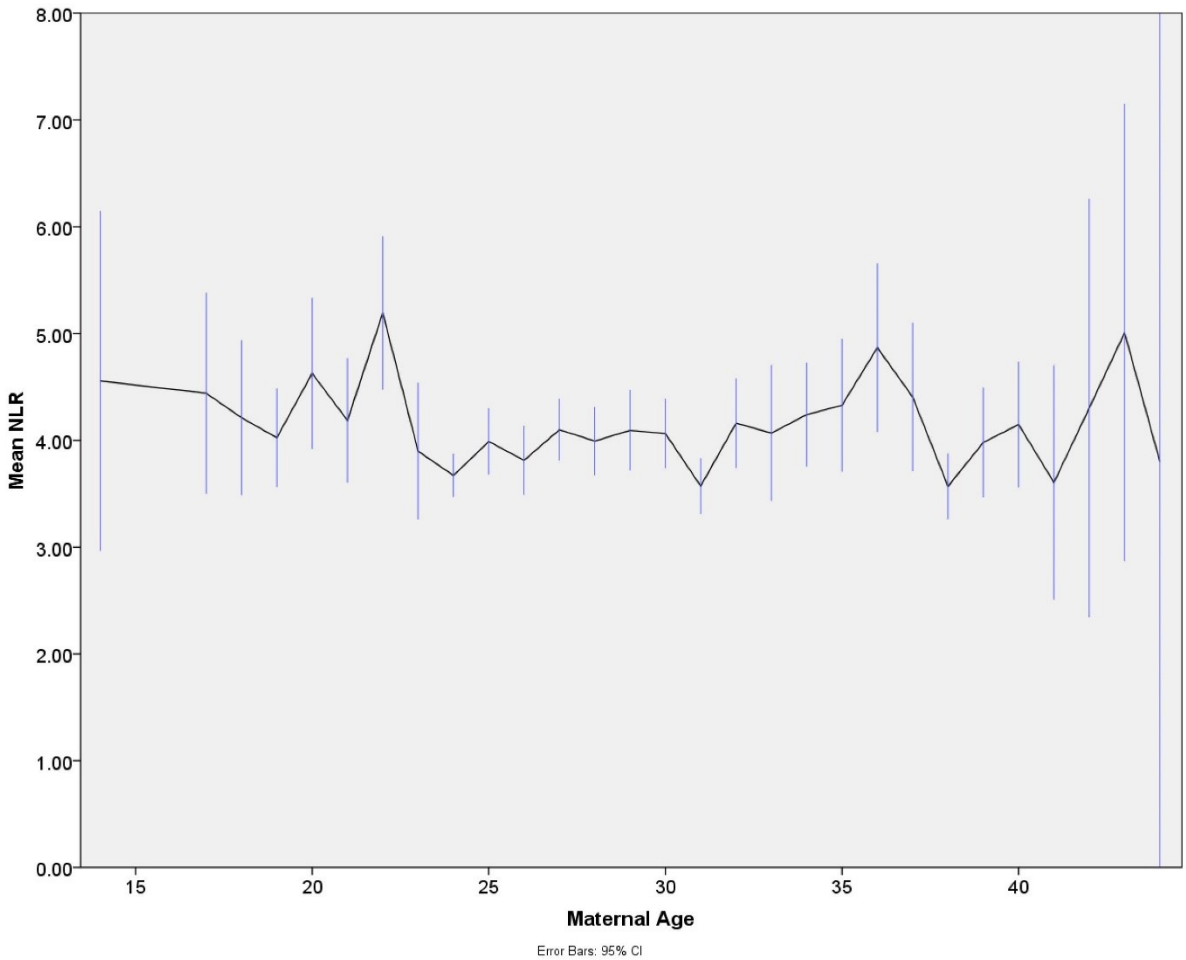

Figure 26: Mean NLR in realtionship with maternal age. The figure was produced by taking all NLR values of all woman of a specific age and producing a mean value.

Figure 26 is a graph of mean NLR versus maternal age. The minimum age was 14 years and the maximum age was 44. As previously shown in Figure 2, the age distribution resembles a bell curve and thus on both ends the confidence intervals show an increase in spread.
Li et al (15) have shown a correlation between age and NLR. Our data suggests there is no association for maternal age and NLR.

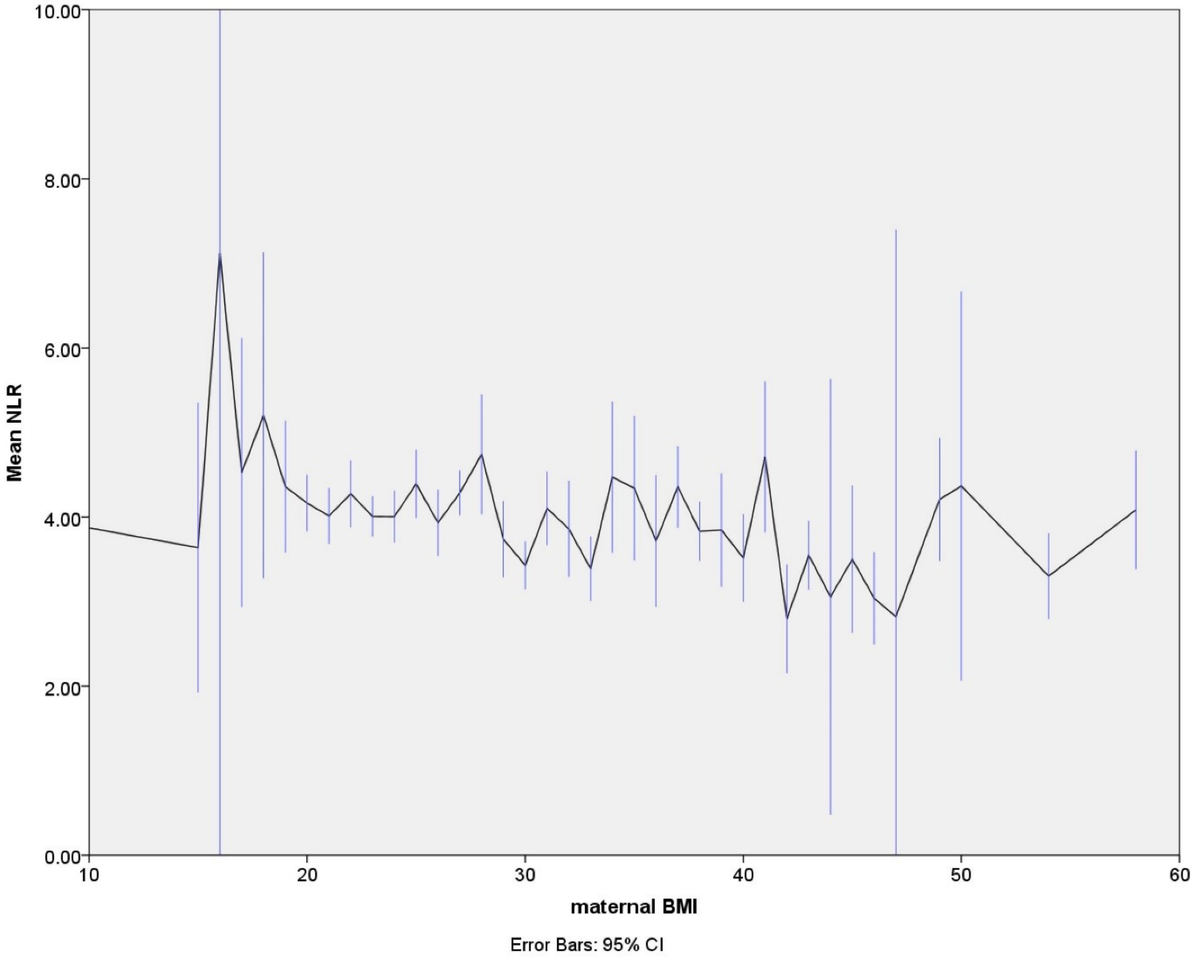

Figure 27: Mean NLR and the maternal BMI

Figure 27 shows the relationship between maternal NLR and maternal BMI at the beginning of pregnancy. BMI was calculated using the first recorded weight in pregnancy and the maternal height. All BMI-calculations were rounded to whole digits.
Several studies within non-maternal populations show increased inflammatory activity in overweight and obese patients. (16-18) This study showed no such link, instead it demonstrated a slight decline in the NLR ratio with an increasing BMI. It seems that the effects the pregnancy has on the immune system causes additional pro inflammatory effects, making it difficult to tease out the effects of obesity.

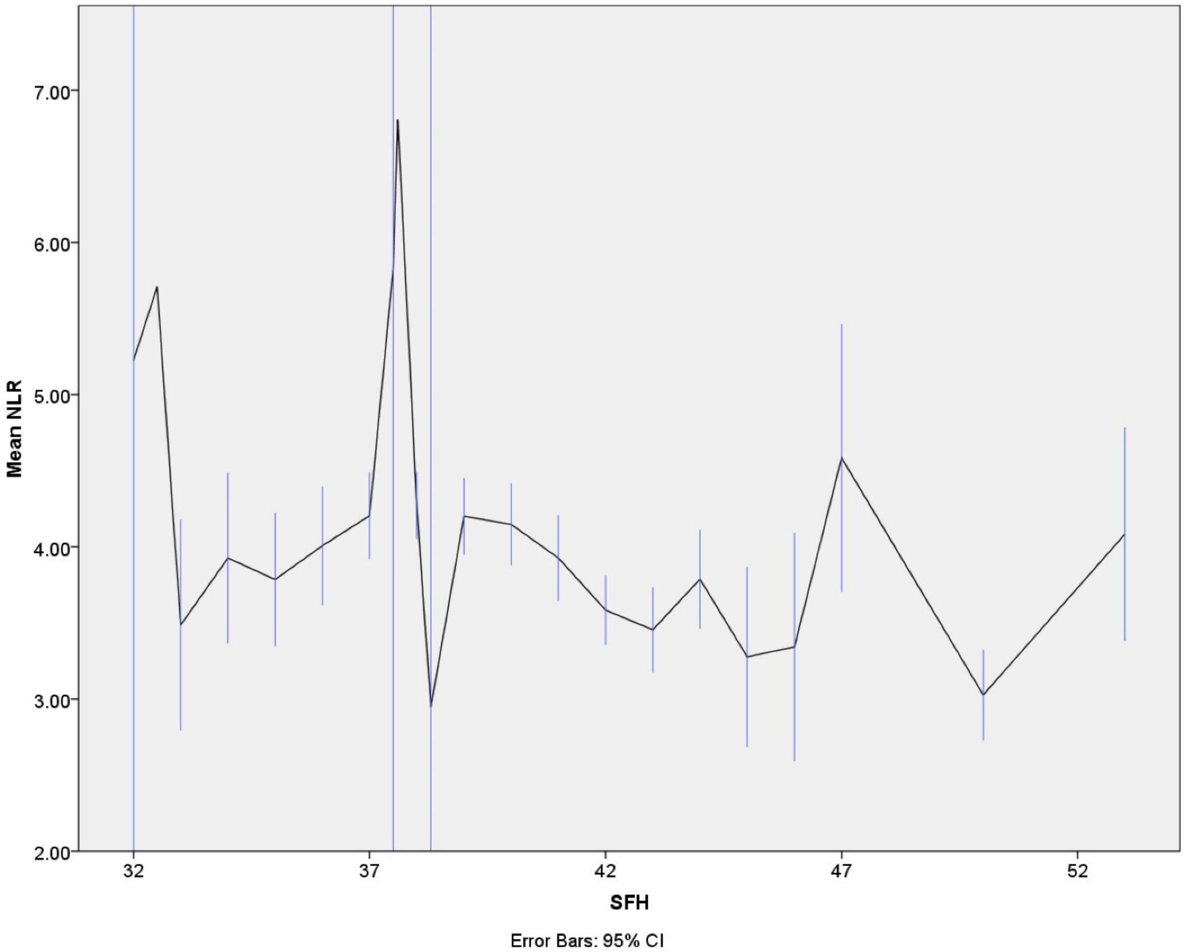

Figure 28: NLR vs Symphysis fundal height

Measuring the symphysis fundal height (SFH) is a traditional method used for detecting small and large for gestational age foetus and in addition polyhydramnios. With the invention and subsequent use of ultrasound it was used less and less. Figure 28 explores the possible link between fundal height and NLR. There appears to be no association between the SFH and the NLR.

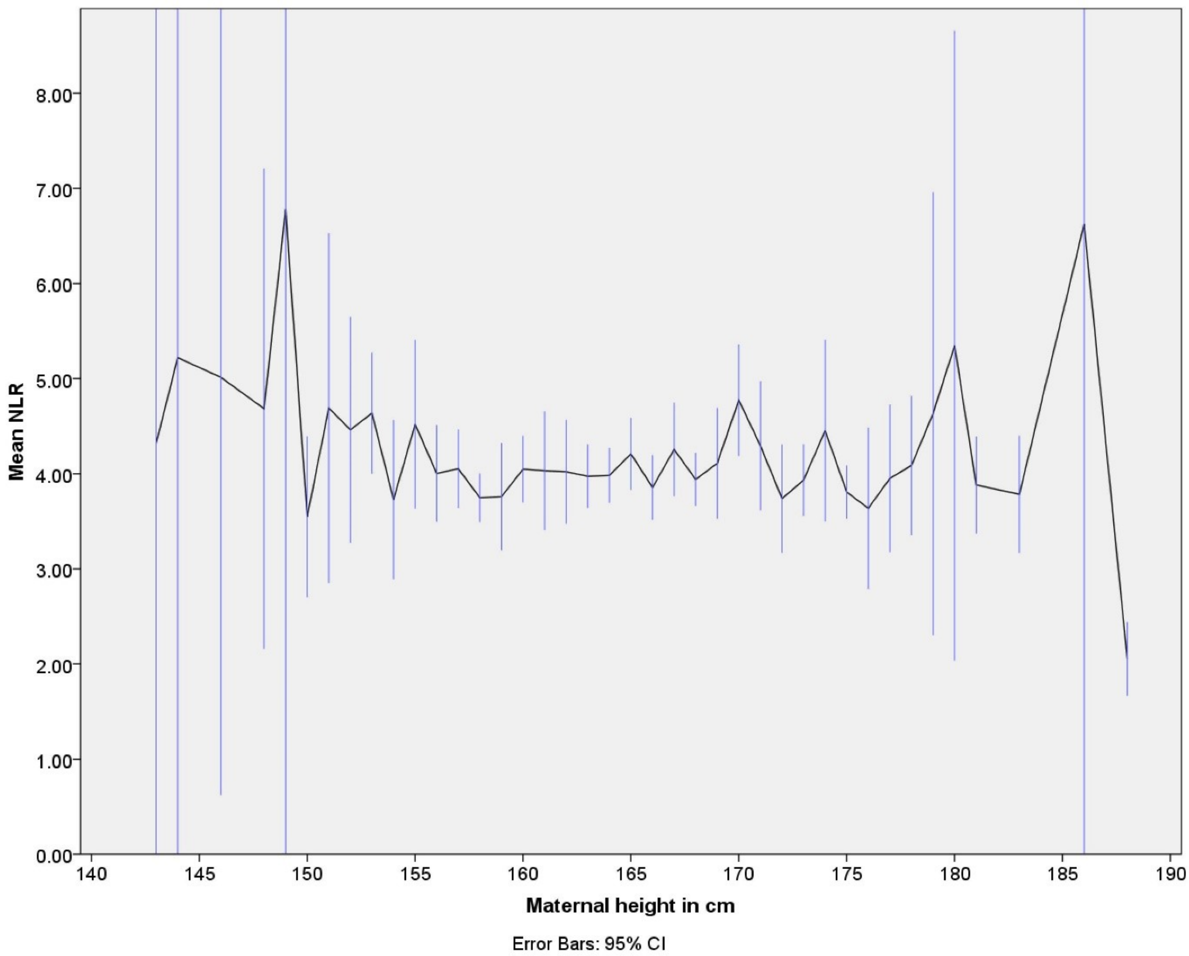

Figure 29: NLR vs maternal height

Figure 29 shows the mean NLR over the maternal height. The range of the height measurements is between 143 cm and 187 cm. There is no clear associations between mean NLR and maternal height visible. As suspected the height of the mothers seems not to have a direct influence to the NLR.

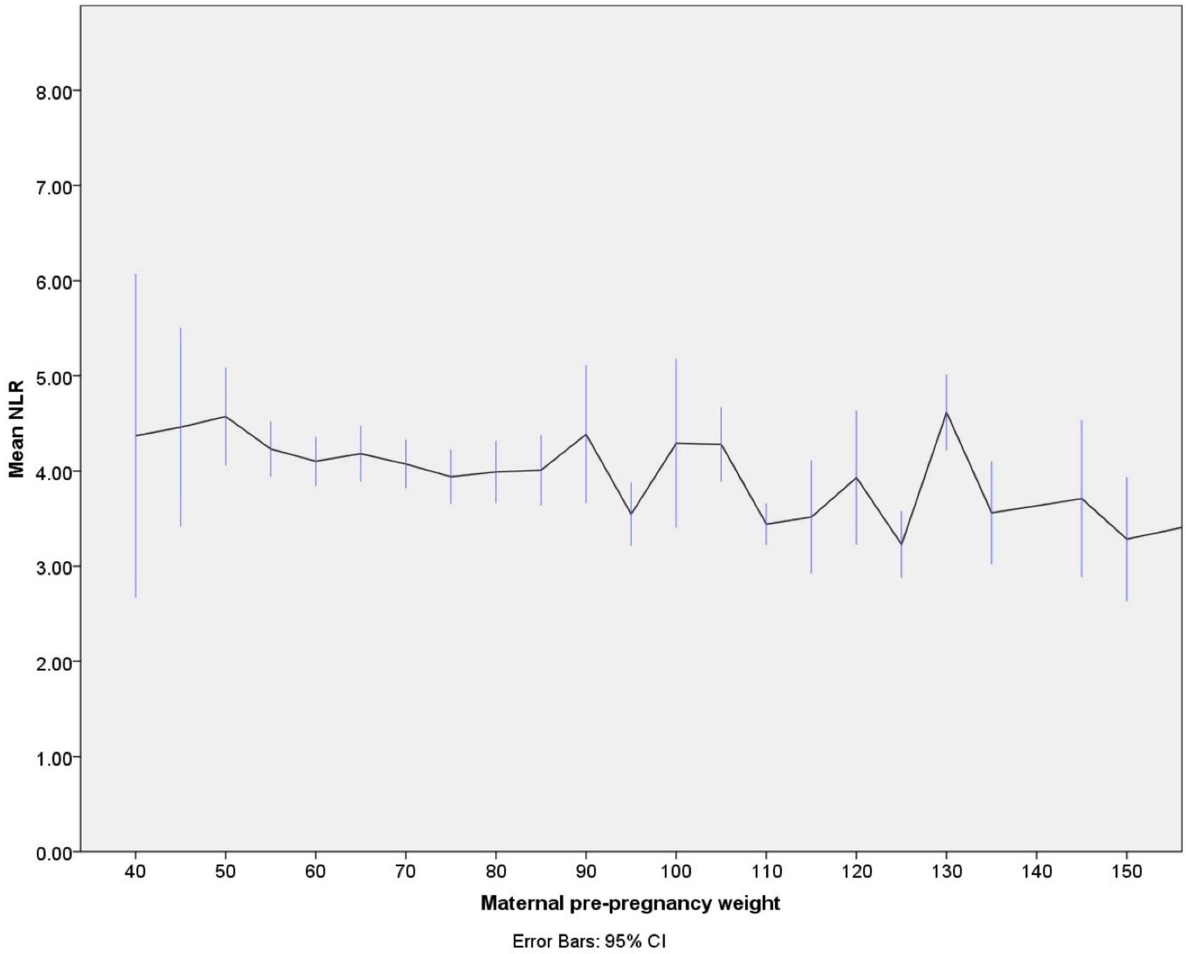

Figure 30: NLR versus the pre-pregnancy weight

As already described in Figure 27 there seems to be no or only a slightly inverse association between the maternal BMI and the maternal NLR. Figure 30 shows the association between maternal pre-pregnancy weight and NLR. As suspected there is a lack of association between the maternal BMI and NLR, there is no correlation between maternal pre pregnancy weight and NLR.

4. Discussion

We found (Chapter 3.3.1) a correlation between the mean number of neutrophil granulocytes and the weeks of gestation. These data confirm that the number of neutrophil granulocytes increase over the course of the pregnancy, from 5.55 ± 1.87 to 8.04 ± 3.16. This occurs despite the physiological hemodilution, that is known to occur with advancing pregnancy.

An indication of this trend could be found in Abbassi-Ghanavati et al (2). However, it has to be noted that Abbassi-Ghanavati et al recorded a normal range with 2.5 and 97.5 percentiles, in order to establish broad normal ranges for clinical use. This dataset is recorded as a mean with standard deviations and therefore more robust against outliers.
The normal range for nonpregnant women was found to be 1.4 to 4.6 but even in the first trimester of the pregnancy this range increased to 3.6 to 10.1 as found by Abbassi-Ghanavati et al (2). This finding is also supported by the result of this study. The data of this study indicate a neutrophil count of 5.55 ± 1.87 in first trimester.

The further findings of this study are a mean of 6.98 ± 2.03 in second and 8.04 ± 3.16 in third trimester. These values were well within the already defined ranges of 3.8 to 12.3 and 3.9 to 13.1 for second and third trimester. Our finding of an increasing neutrophil count can also be seen in Abbassi-Ghanavati et al (2). The cause of the increase of neutrophil granulocytes was not investigated in detail in this study and remains also unknown in the literature.
The increasing neutrophil count could be in preparation for birth and puerperium. Since birth creates an entry point for bacteria heightened immunologic defense mechanisms provide a necessary precaution against bacterial infection. The birth canal is usually contaminated with cervicovaginal bacteria like lactobacilli or the pathogenic Ureaplasma and streptococcus species (19). The extensive wound area after birth and the presence of many different and potentially pathogenic species of bacteria offer much potential for an infection, especially in case of birth canal injuries during birth.

In Chapter 3.3.2 the number of lymphocytes were investigated revealing a reduction in the number of lymphocytes as pregnancy advances. This is not surprising since lymphocytes have a long lifespan and do not seem to be influenced unduly during the course of pregnancy. The decrease in numbers can most likely be attributed to an increased blood volume in late pregnancy. Like the neutrophil granulocytes the lymphocytes are within the given range by Abbassi-Ghanavati et al (2). The normal range given for the first trimester was 1.1 to 3.6 for the second and third trimester the normal range was given at 0.9 to 3.9 and 1.1 to 3.6. In the data collected a mean number was 2.11±0.59 for first trimester and the mean for second and third trimester was 1.90±0.54 and 1.90±0.62.

Unlike the neutrophil granulocytes and their role in preventing bacterial infection, there is little to no risk in transmitting an acute viral infection during and after birth, therefore there is no need for an increase in the number of lymphocytes. Thus there is no increase in the number of lymphocytes over the course of pregnancy.

The NLR showed an increase over the course of pregnancy from a mean of 2.83±1.57 in the first trimester to a mean of 4.77 ±3.37 in the third trimester.

We have shown a positive relationship between NLR and gestational age. Furthermore, it was demonstrated that this is due to an increase in neutrophil granulocytes rather than a decrease in lymphocytes with advancing pregnancy.

As shown in Chapter 3.3.1 there is a notable increase in the frequency of neutrophil granulocytes in the blood. Since the number of lymphocytes in the blood stays roughly the same, this is the reason for the increased NLR levels.

There is a spread and a high number of outliers in Figure 15. There is in addition a notable increase in the spread of the neutrophil count as pregnancy advances. While in the first and second trimester the standard deviation stays roughly the same with 1.87 and 2.03 it shows a notable increase in the third trimester to 3.16. There is also an increase in the range from 13.50 in the first trimester to 14.05 in the second trimester to 26.40 in the third trimester. All these results seem encouraging so that further studies could yield even better results since the cause of this increase in neutrophil granulocytes with advancing pregnancy is unclear and needs further exploration

This study explored also the association between NLR and various neonatal parameters. However, there was no correlation between NLR and length, weight and head circumference of newborns (Figure 23 , Figure 24 and Figure 25). This is most likely due to the exclusion of preterm births, since an inflammatory state has already been shown to have an effect on intrauterine growth restrictions, preterm births and preeclampsia (20).

This study also explored associations between NLR, maternal age and maternal BMI. (Figure 26, Figure 27). No direct association between the maternal age and the NLR was identified. In the literature, it has been shown that age and NLR positively correlate (15). But most likely the differences in age in in the mothers is too small to show an effect. It is surprising that there is not an increase in NLR with an increasing BMI since it has been shown by Bahadir et al, that there is such a link in non-pregnant adults (17). This is most likely due to the pro-inflammatory effect of pregnancy.

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6. Appendices

6.1. Appendix 1

In the following chapter, the maximum number of possible data sets as well as the actual number of data sets and their relations should be estimated.
Taking into account, that 956 women were involved into the study and that the maximum number of blood counts for a woman was ten.

For each blood count 8 values were collected within the blood test at a time in patrticular:

- date of the blood test in relation to the start of the pregnancy,
- absolute number of neutrophil granulocytes,
- absolute number of lymphocytes,
- calculated NLR, absolute number of platelets,
- absolute number of monocytes,
- absolute number of eosinophil granulocytes,
- absolute number of basophil granulocytes

Thus the ideal value for the presented study is:

$$P_{\text{overall}} = \text{Number of woman} \times \text{number of medical investigations} \times \text{number of parameters for each medical investigation}$$

$$P_{\text{overall}} = 956 \times 10 \times 8 = 76480$$

Taking into account, that the expected mothers had on average 3,26 medical examinations during their pregnancy, not counting the 28 women who had no full blood counts, the maximal available parameters for this study are:

$$P_{\text{overall}} = 956 \times 3{,}26 \times 8 = 24930$$

The total number of data points collected was 24.793. This is very close to the above shown estimation and indicates that there were very little missing paramteres.

Statistic basics:

Within this study some statistic expressions are used which should be explained in this chapter:

Average and mean value

There are two different averaging methods common. The resulting value differs. The definitions should be explained in this chapter.
The *average value* used in this study is calculated by summarizing all values and dividing this value by the number of values. The *median* is that value in the ensemble which separates the higher half of the numbers from the lower half. e.g. there is the same amount of data having a higher value and having a lower value. Usually median and average value differ which is shown in Figure 31.

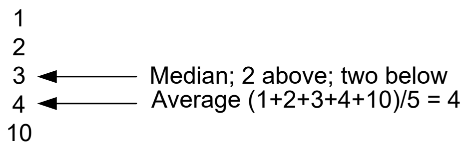

Figure 31: Mean and median value

6.2. Appendix 2

As always in medical investigations (but not only there), the measured values have a more or less great deviation from each other. Values in medical investigations frequently have a large variation. To give an indication of the variation, the standard deviation is a good instrument. The standard deviation characterizes the amount of variation in a set of data values. It is usually denominated by the Greek letter sigma σ or the symbol s.

If the standard deviation is close to 0, then all the data points are very close to the mean value of the data set. A high value for the standard deviation indicates that the data points are spread out over a wide range of values. Figure 32 is an example of this. The exact formula to calculate the standard deviation can be found in the relevant text books.

12		1	
12	Average = 12	5	Average = 12
12	σ = 0	9	σ = 11,3
12		15	
12		30	

Figure 32: Standard deviation

The standard deviation of a set of values describes the variation in the data set, that is, how greatly the values differ from the average value. The standard deviation is also used to select a set of relevant data which excludes runaway data or outlier data. This method is also used in this study.

Normal distribution or Gaussian distribution

The normal or Gaussian distribution is commonly used. It indicates how many samples of a certain value are within the data set. If there is an even distribution around the mean value it is called Gaussian. Another name for it is bell curve. It shows a (theoretical) distribution of a great number of data which have random deviations from the mean values. Normal distributions are useful in statistics for comparison.

Physical quantities that are expected to be the result of independent processes often have distributions that are nearly normal.

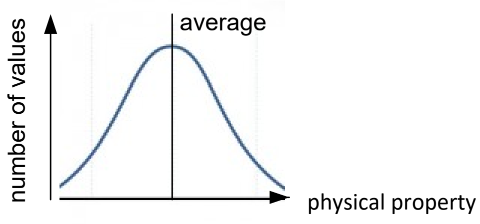

Figure 33: Gaussian or normal distribution

If the distribution is not symmetrical the mathematical treatment is more difficult. The deviation from the symmetry is described as skewness. Such distributions are called Rayleigh distributions.

VII. **Graphs**