CONCLUSIONS

In this thesis, lymphatic maldevelopment as an explanation for the pathophysiology of increased nuchal translucency (NT) was studied. Previous research implicates that an altered or delayed lymphatic development can explain the pathophysiology for increased NT. It can explain both the local and temporary character of increased NT as the jugular lymphatic system undergoes a finalization of its development at the time the NT appears.

We have shown that enlarged jugular lymphatic sacs (JLS) can be visualised with ultrasound in a significant part of fetuses with a normal NT and concluded that the presence of small JLS in cases with normal NT is the result of a normal variation in development. We demonstrated a relation between enlarged JLS and altered jugular vein and ductus venosus Doppler flow velocities in fetuses with increased NT. Altered jugular vein and ductus venosus Doppler flow velocities were demonstrated both in fetuses with and without a cardiac defect. No relation was found between ductus venosus Doppler flow velocities and different types of cardiac defects. We confirmed the abnormal lymphatic endothelial differentiation in fetuses with nuchal edema, the morphological equivalent of increased NT. Furthermore, a possible explanation for the layer of smooth muscle cells which surround enlarged JLS was found. We have also demonstrated an aberrant lymphatic development in fetuses with a normal karyotype. This thesis provides further evidence for an abnormal lymphangiogenesis in fetuses with increased NT.

General discussion and future perspectives

NT measurement in the first-trimester of pregnancy has become a widely used screening method for chromosomal abnormalities. Besides aneuploidy, increased NT is associated with a variety of structural defects and genetic syndromes. A part of the fetuses with enlarged NT present with a normal outcome. The pathogenetic mechanism causing increased NT is, however, not completely understood. Several mechanisms have been suggested such as lymphatic maldevelopment, altered extracellular matrix composition and cardiac failure.

It was suggested that cardiac failure could play a role in the development of increased NT due to the altered ductus venosus Doppler flow velocities and cardiac defects, a frequent finding in fetuses with increased NT. It was hypothesized that a cardiac defect could result in haemodynamic changes by an impaired cardiac function. This might result in a lower velocity of the ductus venosus and subsequently enlargement of the NT.

In this thesis we evaluated if ductus venosus flow alterations in fetuses with increased NT can be explained by haemodynamic changes due to a certain type of cardiac defect. We showed that there are no significant differences in ductus venosus flow between fetuses with increased NT and a certain type of cardiac defect. Furthermore, no differences in intracardiac velocities were found between fetuses with and without increased NT, irrespective of the presence of a cardiac defect. In addition, other signs of cardiac decompensation such as pleural effusion or ascites...
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were not seen in the fetuses with enlarged NT. This is supported by the fact that cardiac defects associated with increased NT like atrioventricular septal defects are not commonly associated with cardiac failure. Huggon et al. also measured intracardiac velocities in first-trimester fetuses and showed no differences between fetuses with normal and increased NT. Our findings are in accordance with a study of Simpson and Sharland who found no differences in types of heart malformations between fetuses with and without increased NT. Furthermore, abnormal ductus venosus flow velocities also have been described in both fetuses with increased and normal NT without a cardiac defect. Thus, we have concluded that ductus venosus flow alterations in fetuses with increased NT cannot be explained by haemodynamic changes due to a certain type of cardiac defect.

Recent studies have demonstrated abnormal lymphatic development as a promising candidate for the pathophysiology of increased NT. Lymphatic development starts in the neck by the formation of the JLS. The JLS normally reorganize into lymphatic nodes after 10 weeks of gestation. Increased NT is suggested to be caused by a delayed or disturbed lymphatic development. A delayed reorganization of the JLS into lymph nodes could explain both transient and regional character of the increased NT. Previous research showed enlarged JLS in both euploid and aneuploid fetuses with increased NT.

Not much was known about the relationship of JLS and normal NT. We prospectively studied the visibility and size of JLS in relation to the size of NT in fetuses with normal NT. We found JLS once or more than once in 32% of the fetuses with normal NT. Enlargement of NT-thickness was associated with a higher probability of the presence of JLS. The mean JLS volumes in fetuses with normal NT were smaller than previously studied JLS volumes of fetuses with increased NT.

The reorganization of the JLS into lymph nodes is a fetus-specific process. This fetus-specific process is also known for the development of the NT and is considered to be a normal variation in development. In most fetuses, the JLS will already have been reorganized into lymph nodes when performing the first ultrasound examination at 11 weeks of gestation. However, the fact that JLS were found in a significant proportion of the fetuses, indicates that in these fetuses the JLS were not yet fully reorganized into lymph nodes during the time of the examination. We suggest that there is a spectrum in lymphatic development. This spectrum varies from a physiological development with visibility of small JLS to a delayed or disturbed lymphatic development with visibility of large JLS.

We further assessed jugular lymphatic development in relation to haemodynamics in the fetal neck, heart and ductus venosus to see whether dilation of the JLS affects the haemodynamics in these vessels. We demonstrated altered jugular vein and ductus venosus Doppler flow velocities in fetuses with increased NT and enlarged JLS. Visibility of JLS was related to a higher pulsatility index for veins (PIV) and lower velocity during atrial contraction (a-V) of the ductus venosus. Larger JLS volumes were associated with a higher jugular vein and ductus venosus PIV and a lower jugular vein and ductus venosus a-V. Martinez et al. evaluated carotid artery and
jugular vein Doppler flow in fetuses with normal and increased NT and found no differences between the two groups.24 This was, however, a cross sectional study with only 22 fetuses with increased NT.

The jugular vein has an anatomic relation with the developing JLS as drainage of the lymphatic fluid takes place through the jugular vein. We hypothesize that the higher jugular vein PIV in fetuses with increased NT could be the result of an increased amount of lymphatic fluid in enlarged JLS of these fetuses. This could cause a higher drainage through the jugular vein and might result in a higher venous pressure, thus explaining the finding of a higher jugular vein PIV in fetuses with increased NT. To gain more insight in the abnormal flow velocities in the jugular vein it would be interesting to investigate if there is a temporal relation between the development of the JLS and abnormal flow velocities in the jugular vein in the individual fetus. A similar temporal relation has also been described before between increased NT and JLS.18 Due to the small size of our study it was not possible to examine this possible relation.

A disturbance in lymphatic development can explain both the local and temporary character of increased NT. Our findings support the hypothesis of disturbance in lymphatic development. Previous morphological research in aneuploid fetuses with nuchal edema, which is the morphological equivalent of increased NT19, demonstrated an abnormal endothelial differentiation of the enlarged JLS.21 We confirmed this abnormal endothelial differentiation and found blood vessel characteristics including an increased expression of Vascular Endothelial Growth Factor (VEGF)-A and Neuropilin (NP)-1 and a diminished expression of lymphatic markers Prox-1 and Podoplanin.21 We further assessed the endothelial differentiation of trisomy 21 fetuses with nuchal edema and enlarged JLS and found a decreased expression of FOXC-2 and increased expression of platelet derived growth factor (PDGF)-B. This could explain the presence of smooth muscle cells surrounding the enlarged JLS. Furthermore, we demonstrated increased expression of Sonic hedgehog (Shh) in the lymphatic endothelial cells of the JLS of trisomy 21 fetuses, which acts upstream of VEGF.25,26

Previous morphological research of fetuses with increased NT has focused on the lymphatic development of aneuploid fetuses. We were the first to assess the endothelial differentiation of first- and second trimester euploid fetuses, including fetuses with Noonan syndrome. This is a developmental disorder associated with increased NT.27 In 60-70% of the cases, a gene mutation can be demonstrated. We confirmed the findings of abnormal endothelial differentiation in these fetuses as described previously.21 However, we evaluated a small and heterogeneous group of first- and second trimester fetuses including Noonan syndrome fetuses with several different gene mutations. Furthermore, all assessed fetuses showed a markedly increased NT. Therefore, we possibly evaluated fetuses at the end of the spectrum of abnormal lymphatic development.

A disturbed endothelial differentiation could be related to the lymphatic abnormalities, altered jugular vein and ductus venosus flow found in fetuses with increased NT. Previous research in trisomy 16 mouse embryos, an animal model for trisomy 21, showed a disturbed interaction
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between endothelial and neuronal pathways in the JLS, aortic arch and ductus venosus region. An abnormal innervation of the jugular vein and ductus venosus could attribute to the abnormal flow velocities in fetuses with increased NT which previously has been suggested for the ductus venosus.

This thesis demonstrates that there is a disturbance in endothelial differentiation in both euploid and aneuploid fetuses with increased NT. Further research of the genes involved with aberrant lymphatic endothelial differentiation is needed. For example, an in vivo mouse model could be created in which endothelial differentiation can be investigated prospectively. In this model, the endothelial differentiation possibly could be influenced. In this way, more information of the genes involved in development of an abnormal endothelial differentiation and subsequent nuchal edema could be gathered.

Abnormal endothelial processes, such as an altered expression of VEGF-A, NP-1 and Shh, have also been described to play a role in relation to development of cardiovascular defects. These alterations could explain the frequently found cardiac defects in fetuses with increased NT. To assess the endothelial differentiation of the developing heart, further research in a mouse model such as the trisomy 16 mouse is needed. This way, the endothelial differentiation can be evaluated in an earlier stage of cardiovascular development.

NT measurement has become an established screening method to detect fetuses at risk for a chromosomal abnormality. Fetuses with a normal karyotype are still at risk for a structural defect or genetic syndrome. In the future, a screening model could be developed to investigate the chance of an adverse outcome in euploid fetuses with increased NT. In this model NT, JLS volumes, jugular vein and ductus venosus should be measured at 12-13 weeks’ gestation. An anomaly scan should be performed at 14-15 weeks’ gestation when fetal structures such as the heart and outflow tracts are more visible. The JLS, jugular vein and ductus venosus should also be measured again at this gestation. Therefore, larger datasets of JLS, jugular vein and ductus venosus flow measurements of fetuses with normal and increased NT should be collected to create normal values. Furthermore, to implement jugular vein and ductus venosus Doppler measurements in this screening model, the inter- and intraobserver variabilities of these vessels need to be evaluated.

In monochorionic twin fetuses with increased NT, a more than three-fold increase in risk for twin-to-twin transfusion syndrome (TTTS) development has been described. Monochorionic twin fetuses are characterized by the presence of vascular anastomoses between the two fetoplacental circulations. The pathophysiologic mechanism has been suggested to be cardiac dysfunction due to hypervolaemic congestion in the recipient twin. We showed that NT measurement can also be used as a screening method to predict TTTS and might be considered for all monochorionic twins as TTTS screening tool besides aneuploidy screening. Interestingly, a recent study also demonstrated abnormal ductus venosus velocities in fetuses with NT discordance. No studies have been performed to evaluate the jugular lymphatic development in these fetuses. It would be interesting to investigate the haemodynamics
including the jugular vein, ductus venosus and intracardiac velocities in relation to the jugular lymphatic development in monochorionic pregnancies to gain more insight in the pathophysiological mechanism of increased NT in these fetuses.

In conclusion, this thesis gives further evidence for a disturbance in lymphatic development in fetuses with increased NT. Future research should focus on the associated findings of increased NT such as cardiac defects, altered jugular vein and ductus venosus Doppler flow velocities, and the possible causes of altered endothelial differentiation.
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REFERENCES

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