**SUMMARY**

The nuchal translucency (NT) is a translucent area in the neck region of the first-trimester fetus which can be visualised by using ultrasound. It usually disappears after 14 weeks’ gestational age. NT measurement is a widely used screening method for chromosomal abnormalities. Besides aneuploidy, increased NT is associated with structural defects such as cardiac defects and genetic syndromes. A part of the fetuses with increased NT have a normal outcome. Despite the worldwide use of NT measurement as a screening tool, the pathophysiology of increased NT is not completely elucidated. Several theories such as cardiac dysfunction and abnormal lymphatic development have been proposed. Cardiac failure has been suggested because of altered ductus venosus Doppler flow velocities and cardiac defects, a frequent finding in fetuses with increased NT. Recent studies have focused on a disturbance in lymphatic development as explanation for increased NT. The development of the lymphatic system starts in the neck by the formation of the jugular lymphatic sacs (JLS). The JLS normally reorganize into lymphatic nodes after 10 weeks of gestation. The formation of the lymphatic system is completed by the ingrowth of the right thoracic duct into the left JLS. This forms the main site of lymphatic drainage into the systemic circulation. Consequently, the fluid in the neck can drain away. A disturbed lymphangiogenesis can explain both the local and temporary character of the increased NT as it appears when the jugular lymphatic system undergoes its finalization. Fetuses with increased NT demonstrate enlarged JLS on ultrasound. Development of the increased NT is related to JLS volume, with NT expansion preceding the JLS enlargement. In this thesis, lymphatic development in fetuses with normal and increased NT was further assessed. Also, haemodynamics in relation to increased NT and lymphatic development was evaluated.

**Chapter 1** gives an overview of the different theories of the pathophysiology of increased NT and describes the outline of the thesis. In **chapter 2** the ultrasonographic appearance of the JLS in first-trimester fetuses with a normal NT was assessed. Seventy-five fetuses with a normal NT (< 95th percentile) were examined weekly between 11 and 17 weeks of gestation. In 24 (32%) of the 75 fetuses, the JLS could be observed once or more than once. Enlargement of the NT thickness was associated with a higher probability of presence of the JLS. Gestational age was predictive for the presence of the JLS, with the highest probability between 13 to 15 weeks of gestation. The mean volumes of the JLS found in these fetuses were smaller than those previously described in fetuses with an increased NT. We conclude that there is a spectrum in lymphatic development which varies from a physiological development with visibility of small JLS to a delayed or disturbed lymphatic development with visibility of large JLS. **Chapter 3** evaluates blood flow in relation to jugular lymphatic distension in fetuses with normal and increased NT. Seventy-two fetuses with normal NT and 71 fetuses with increased
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NT were evaluated. NT-size, JLS, jugular vein and ductus venosus pulsatility index for veins (PIV), velocity during late diastole (a-V), and intracardiac velocities were measured. JLS were visualized in 22/72 (31%) fetuses with normal and in 55/71 (77%) fetuses with increased NT. A higher PIV and lower a-V of the jugular vein and ductus venosus were found in fetuses with increased NT compared to fetuses with a normal NT. Visibility of JLS was associated with a higher PIV and lower a-V of the ductus venosus, but not with an altered jugular vein flow. Larger NT and larger JLS volumes were associated with higher PIV and lower a-V of the jugular vein and ductus venosus. No significant differences were found in intracardiac velocities between fetuses with normal and increased NT, irrespective of a cardiac defect. This study shows a relation between increased NT, jugular lymphatic distension and altered blood flow in jugular vein and ductus venosus. The fact that no differences were found in intracardiac velocities suggests that cardiac failure cannot explain the finding of an increased NT and associated abnormal Doppler flow in the jugular vein and ductus venosus. This was further supported by the findings described in chapter 4. In this chapter, the ductus venosus flow velocities and a possible relation with the type of cardiac defect in fetuses with increased NT was evaluated. Seventy-two fetuses with a normal NT and 137 fetuses with increased NT were evaluated. The ductus venosus PIV, ductus venosus a-V, and intracardiac velocities were evaluated. A cardiac defect was found in 45 fetuses with increased NT. Fetuses with increased NT showed a higher ductus venosus PIV and lower a-V compared to fetuses with a normal NT. Within the group of fetuses with an increased NT, a higher PIV and lower a-V were found in case of a cardiac defect compared to cases with a normal heart. No differences in PIV and a-V were found between the types of cardiac defects. Intracardiac velocities showed no differences between fetuses with normal and increased NT, irrespective of the presence of a cardiac defect. Therefore we conclude that altered ductus venosus flow velocities found in fetuses with an increased NT can not be explained by cardiac failure due to a specific altered cardiac anatomy.

Previous morphological research showed nuchal edema and enlarged JLS in aneuploid fetuses with increased NT, together with abnormal lymphatic endothelial differentiation. This was indicated by decreased expression of the lymphatic markers Prox-1 and Podoplanin. Increased expression of blood vessel markers, including vascular endothelial growth factor (VEGF)-A and Neuropilin (NP)-1 were found. The enlarged JLS contained erythrocytes and were surrounded by smooth muscle cells. In chapter 5 we confirmed this abnormal endothelial differentiation in aneuploid fetuses with increased NT. Assessment of the endothelial differentiation showed a decreased expression of FOXC-2 and increased expression of platelet derived growth factor (PDGF)-B. This could explain the smooth muscle cells surrounding the enlarged JLS. Furthermore, we demonstrated increased expression of Sonic hedgehog (Shh) which acts upstream of VEGF in the lymphatic endothelial cells of the JLS of these fetuses.

Earlier studies have focused on the lymphatic development of aneuploid fetuses with increased NT. Chapter 6 describes the lymphatic development of 7 euploid first- and second trimester fetuses with increased NT, including fetuses with Noonan syndrome. This is a developmental
disorder associated with increased NT. The characteristics of this syndrome include hypertelorism, a downslant of the eyes, low-set ears, a short stature, congenital heart defects and a mild mental retardation. We demonstrated an abnormal endothelial differentiation in these euploid fetuses as previously shown in aneuploid fetuses with increased NT. In 60-70% of the Noonan syndrome cases, a gene mutation can be demonstrated. Noonan syndrome is caused by mutations in the \textit{PTPN11} gene in approximately 50% of cases. Mutations in the \textit{SOS1}, \textit{RAF1}, \textit{MEK1} and \textit{KRAS}-gen also have been associated with Noonan syndrome. In \textbf{chapter 7} three cases of euploid fetuses with increased NT are described where analysis of the \textit{PTPN11} en \textit{KRAS} gene led to detection of a de novo mutation and the prenatal diagnosis of Noonan syndrome. We conclude that in case of increased NT and a normal karyotype, Noonan syndrome mutation detection needs to be considered.

Measurement of the NT is used as a screening method to detect fetuses at risk for aneuploidy. In \textbf{chapter 8} we demonstrated that NT measurement can also be used as a screening method to predict twin-to-twin transfusion syndrome (TTTS). TTTS develops in 15% of all monochorionic twin pregnancies. Without intervention, TTTS often leads to either severe morbidity mostly associated with preterm birth or demise of one or both fetuses. A total of 61 monochorionic twins were enrolled. We found a positive predictive value of 50% and negative predicted value of 86% for TTTS development if NT discordance was 20% or more. We conclude that NT discordance of more than 20% in monochorionic twins is associated with an increased risk for subsequent development of TTTS, and earlier presentation of symptoms. Standardized NT measurements might be considered for all monochorionic twins as TTTS screening tool besides aneuploidy screening.

A general conclusion and future perspectives are given in \textbf{chapter 9}. With this thesis we give further evidence for the hypothesis of a disturbance in lymphatic development in fetuses with increased NT. Also, we show that cardiac failure cannot explain the temporary and local phenomenon of 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.