INTRODUCTION

In the first trimester of pregnancy, the fetal neck contains subcutaneous fluid that can be visualised by ultrasonography as nuchal translucency (NT). The ultrasonographic measurement of NT is a widely used and sensitive screening method for trisomy 21 with a detection rate of 90% for a 5% false-positive rate.\(^1\)\(^-\)\(^3\) Increased NT is associated with trisomy 21 and other chromosomal abnormalities like trisomy 18, trisomy 13 and monosomy X.\(^2\) Fetuses with increased NT and a normal karyotype are still at risk for various structural anomalies, mainly cardiac defects, and several genetic syndromes.\(^4\)\(^,\)\(^5\) However, some of the fetuses with increased NT are born alive and healthy.\(^6\)

Pathophysiology

Despite the worldwide use of NT measurement as a screening method, the pathophysiology of increased NT is not completely elucidated. Several theories such as cardiac dysfunction, alterations in the extracellular matrix and abnormal lymphatic development have been proposed. Recent studies point towards a disturbance in lymphatic development as this can explain both temporary and regional character of increased NT. All proposed theories will be evaluated in the following paragraphs.

I. Cardiac dysfunction

Cardiac defects

Cardiac failure has often been suggested to play a role in the development of increased NT due to the frequently found cardiac defects.\(^7\)\(^,\)\(^8\) It was hypothesized that a cardiac defect could result in increased end-diastolic right ventricular pressure and subsequent development of nuchal edema.\(^9\) However, not all fetuses with increased NT have a cardiac defect. Septal defects, the most frequently found cardiac defects in fetuses with increased NT\(^7\)\(^,\)\(^10\) are not commonly associated with haemodynamic compromise during fetal life. A study of Simpson and Sharland demonstrated a wide variety of cardiac defects with different haemodynamic effects in both fetuses with a normal and increased NT.\(^11\) No differences in types of heart malformations between fetuses with and without increased NT were found.\(^11\) Morphological research found higher levels of both atrial natriuretic peptide and brain natriuretic peptide in trisomic fetuses with increased NT, which is associated with cardiac failure in postnatal life.\(^12\)\(^,\)\(^13\) Another morphological study, however, evaluated calcium ATPase in cardiac tissue of trisomic fetuses, which is also known to be related with postnatal heart failure. No significant decrease in calcium ATPase expression was found compared to controls.\(^14\)
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Ductus venosus
Several Doppler studies have been performed to assess haemodynamics in fetuses with increased NT. These studies demonstrated a changed Doppler flow pattern of the ductus venosus in first trimester fetuses with increased NT. Montenegro et al. suggested that this may indicate a defective atrial contraction or ventricular compliance and indirectly reflects cardiac failure. A changed Doppler flow pattern of the ductus venosus has also been demonstrated in second- and third-trimester fetuses with overt cardiac failure. The underlying cardiac defects in fetuses with increased NT were suggested to play a role in cardiac failure. The fact, however, that abnormal ductus venosus flow velocities are also found in aneuploid fetuses with increased NT and a normal heart does not support the theory of cardiac failure as an explanation for increased NT. The finding of abnormal ductus venosus flow velocities in fetuses with normal NT with and without a cardiac defect also suggests that increased NT cannot be explained solely by haemodynamic changes due to cardiac abnormalities.

A morphological study of the ductus venosus region of trisomy 16 mouse embryos, which is considered to be an animal model for human trisomy 21, was performed to investigate if the altered ductus venosus velocities in fetuses with increased NT could be caused by local changes in morphology or development of the ductus venosus. The ductus venosus sphincter contains neural crest derived adrenergic nerves, causing contraction and relaxation, which could influence the blood flow. Thickening of the endothelium of the ductus venosus and upregulation of Neural Cell Adhesion Molecule (NCAM) was observed in the trisomy 16 embryos. NCAM plays a role in vasculogenesis and neurogenesis and affects neural crest migration. Alterations of the endothelium could explain the abnormal ductus venosus flow velocities in fetuses with increased NT. NCAM upregulation was not related to a specific cardiac morphology and therefore also could explain altered ductus venosus Doppler flow velocities in fetuses having increased NT without a cardiac defect.

Intracardiac velocities
Several studies have been performed to assess cardiac function in fetuses with increased NT by measuring Doppler flow velocities across the atroventricular (AV)-valves. Peak flow velocities in early diastole (E) and late diastole with atrial contraction (A) were measured. Rizzo et al. demonstrated a significant lower E-wave of both the mitral and tricuspid valves compared to controls which was explained to be a possible reduction in myocardial relaxation of these fetuses. However, they evaluated only euploid and structurally normal fetuses between 20-23 weeks of gestation who were diagnosed with increased NT in the first trimester which had been resolved in all but two cases. Also, no relation between the size of the NT and the E-wave values was found. Furthermore, their findings are not in accordance with the altered ductus venosus flow velocities in first trimester fetuses with increased NT as specifically changes in the A-wave should have been expected. Two other studies evaluated the cardiac function when the increased NT was still present. Huggon et al. found no differences in intracardiac
velocities between normal fetuses and euploid fetuses with increased NT. Haak et al. assessed first-trimester fetuses with a normal and increased NT, either euploid or aneuploid and with or without a cardiac defect, and found no differences in intracardiac velocities between the two groups.

Zoppi et al. investigated the Doppler velocity waveforms of the left atrioventricular valve of 285 fetuses with increased NT. They demonstrated a different Doppler flow pattern in trisomy 21 fetuses with the lowest E-wave velocity crossing the baseline in at least one of the examined profiles. This was suggested to be an expression of greater filling pressure or altered relaxation of the ventricle. However, the Doppler flow measurements were not evaluated in fetuses with a normal NT. Also, the findings could not be related to the presence of a cardiac defect because post mortem examination of the terminated pregnancies was not performed.

Regurgitation across the tricuspid valve is highly associated with aneuploidy. Regurgitation is substantially higher in fetuses with than those without a cardiac defect. The prevalence of tricuspid regurgitation increases with the size of the NT. It disappears with the disappearance of increased NT. It was suggested that increased cardiac pre- or afterload could be related to the development of tricuspid regurgitation. Against this is the lack of right ventricular dilatation which could be expected if there were volume overload.

In conclusion, many studies have been performed to assess cardiac failure due to a cardiac defect as a possible cause for increased NT. However, not all fetuses with increased NT have a cardiac defect, other signs of cardiac decompensation are rarely seen in fetuses with an enlarged NT and it cannot explain the local fluid accumulation in the neck region. Therefore, cardiac failure does not seem to explain the pathophysiology of increased NT.

II. Overperfusion
It was also hypothesized that increased NT could be caused by an overperfusion of the fetal head due to preferential blood flow to the head and neck as a result of an obstruction in the aortic arch. Hyett et al. found a narrowing of the aortic isthmus with widening of the ascending aorta in 55% of 60 studied trisomy 21 and 36% of 29 studied trisomy 18 fetuses. In 17 trisomy 13 and monosomy X fetuses, however, narrowing of the aortic isthmus was accompanied by narrowing of the ascending aorta. As the ascending aorta in these fetuses was relatively hypoplastic, the increased NT cannot be explained by an overperfusion of the fetal head. Also, the fact that narrowing of the aortic isthmus was only demonstrated in a part of the fetuses with increased NT, does not support the theory that increased NT is a direct consequence of a cardiac defect.

Martinez et al. evaluated the carotid artery and jugular vein Doppler flow velocity waveforms and found no differences in carotid artery or jugular vein pulsatility index of fetuses with increased NT compared to fetuses with normal NT. They concluded that overperfusion or venous congestion of the head is not a likely causative mechanism involved in increased NT.
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Venous congestion in the head and neck could result from constriction of the fetal body such as congenital diaphragmatic hernia and esophageal atresia.\textsuperscript{38,39} In a wide range of skeletal dysplasias, increased NT may be primarily explained by venous congestion of the fetal head and neck or impaired cardiac function due to intrathoracic pressure.\textsuperscript{40}

III. Altered extracellular matrix

Local changes in the extracellular matrix of the nuchal region have been suggested as an underlying mechanism for increased NT.

Composition of the extracellular matrix

It was found that in trisomy 21 fetuses, collagen VI forms a dense, irregularly arranged mass reaching from the epidermis to the subcutis of the nuchal skin whereas in normal controls collagen VI was restricted to the upper region of the epidermis.\textsuperscript{41} Collagen VI is known to bind hyaluronan, a glycosaminoglycan of the extracellular matrix.\textsuperscript{42} Hyaluronan was found abundantly in the skin of trisomy 21 fetuses, in contrast to the small amount found in the normal controls.\textsuperscript{41,43} The authors suggested that collagen VI bound hyaluronan could affect the process of water binding in the skin leading to increased NT.\textsuperscript{41,43}

Trisomy 13, trisomy 18 and Turner syndrome fetuses, however, did not show differences in hyaluronan amount compared with controls.\textsuperscript{44} Also, the alterations in the extracellular matrix of the nuchal skin of the trisomy 21 fetuses were similar to the alterations in samples of the skin of the fetal leg. Another study investigated distribution patterns for a number of glycosaminoglycans in the nuchal skin of normal and chromosomally abnormal fetuses and found no differences in glycoproteins between trisomy 21,18 and 13 fetuses compared with controls.\textsuperscript{45}

Von Kaisenberg et al. evaluated the composition of the dermal connective tissue of trisomy 21,18,13 and normal fetuses and confirmed the abundant collagen VI in trisomy 21 fetuses.\textsuperscript{46} No cavities were demonstrated in the nuchal skin which is in contrast to a recent study in which cavities were found in the nuchal edema of the evaluated trisomy 21 fetuses.\textsuperscript{47} Collagen I fibers were more widely spaced in the skin than in normal fetuses.

In trisomy 18 fetuses, the dermis contained fluid-filled cavities. The collagen fibers, predominantly collagen III, were thinner and shorter. In trisomy 13 fetuses, the epidermis and dermis contained fluid-filled cavities. The collagen fibers alternated between loosely arranged areas with little precipitate and more dense areas with intense staining and excess of collagen III and VI. It was concluded that the alterations in the extracellular matrix could only partially be explained by single gene dosis effects of genes coding for matrix components on the extra chromosomes 21, 18 and 13.\textsuperscript{46}

COL6A1 and COL6A2

Collagen VI expression is regulated by genes (COL6A1 and COL6A2), which are located on chromosome 21.\textsuperscript{41,48} Quarelllo et al. investigated the genes encoding for COL6A1 and 2 in
trisomy 21 fetuses with and without increased NT.\(^49\) They found overexpression of COL6A1 and 2 compared to normal fetuses, irrespective of their initial NT thickness and gestational age. Therefore, the authors stated that the fetal morphology corresponding to enlarged NT may not exclusively be explained by the overexpression of these genes.\(^49\) Research of the nuchal skin of trisomy 16 mouse embryos demonstrated wide intercellular spaces with increase in precipitate containing glycosaminoglycans which is in accordance with the findings in trisomy 21 fetuses.\(^46\) This cannot be the effect of overexpression of collagen VI as the encoding genes COL6A1 and 2 are located on the mouse chromosome 10.

In summary, immunohistochemical studies of the skin of aneuploid fetuses have demonstrated specific alterations of the extracellular matrix in each trisomy which have been attributed to gene dosage effects. The extracellular matrix of euploid fetuses with increased NT, however, has not been investigated. Also, an altered extracellular matrix does not explain the temporary character of the NT and does not seem to explain the wide range of abnormalities associated with increased NT.

**IV. Abnormal lymphatic development**

The first time malformations of the lymphatic system were described, was in spontaneously aborted fetuses with cystic hygroma.\(^50,51\) Morphological research of the nuchal region and lymphatic morphology of fetuses with monosomy X, trisomy 21, trisomy 13 and euploid fetuses demonstrated numerous and dilated lymph vessels in the trisomic and euploid fetuses, and absent or hypoplastic vessels in monosomy X fetuses.\(^52\)

Morphological studies of both trisomy 16 mice embryos and human fetuses demonstrated that the nuchal region of fetuses with increased NT is mesenchymal edema containing nuchal cysts and is accompanied by distension of the jugular lymphatic sacs (JLS).\(^53,54\) Several studies have shown that enlarged JLS can be visualised by ultrasound in the majority of both aneuploid and euploid fetuses with increased NT.\(^53,55,56\) A longitudinal ultrasound study of fetuses with increased NT demonstrated that the development of the NT is related to the volume of the JLS, in which NT expansion preceded the JLS enlargement.\(^57\) The lymphatic development starts in the neck by the formation of the JLS. Bilaterally, buds of lymphatic endothelial cells arise from the internal jugular veins, which fuse and form the JLS. The peripheral lymphatic system is formed by sprouting from these sacs.\(^58-60\) The JLS normally reorganize into lymphatic nodes after 10 weeks of gestation.\(^59\) The formation of the lymphatic system is completed by the ingrowth of the right thoracic duct into the left JLS, thereby connecting several lymphatic vessels, and forming the main drainage site of lymphatic fluid into the systemic circulation.\(^58\) Consequently, the fluid in the neck can drain away.

Further research of the jugular lymphatic system showed an aberrant lymphatic endothelial differentiation in aneuploid human fetuses with increased NT and trisomy 16 mouse embryos.\(^61\) A diminished expression of the lymphatic markers Prox-1 and Podoplanin and blood vessel
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characteristics including increased Neuropilin (NP)-1 and Vascular Endothelial Growth Factor (VEGF)-A expression were demonstrated. Overexpression of VEGF-A has been described to lead to hypervascularization, increased vascular permeability and edema.\textsuperscript{62,63} VEGF-A and NP-1 are also involved in cardiovascular development and therefore could play a role in the development of cardiovascular abnormalities which are frequently found in fetuses with increased NT.\textsuperscript{64,65} In addition, the enlarged JLS were surrounded by smooth muscle cells, which is normally only observed in blood vessels or large collecting lymph vessels.\textsuperscript{66} Also, erythrocytes were found in the enlarged JLS which is an abnormal finding in lymphatic vessels.\textsuperscript{67} In the edematous nuchal subepidermis, several dilated hyperplastic lymph vessels were observed. An aberrant endothelial differentiation could be an explanation for enlarged JLS with subsequent development of increased NT. Furthermore, an abnormal endothelial differentiation provides a link to the cardiovascular defects in fetuses with increased NT, as endothelial development plays a role in cardiac development.\textsuperscript{68,69}

Morphological studies of Turner syndrome fetuses also demonstrate an abnormal lymphatic development. In these fetuses, hypoplastic vessels were found in the subcutaneous mesenchymal tissue.\textsuperscript{47} Fetuses with Turner syndrome usually present with a massively increased NT, which is referred to as ‘cystic hygroma’. The bilateral cavities in the posterior neck have been suggested to be distended and hyperplastic JLS, which failed to connect with the venous system.\textsuperscript{52} Aplasia of the JLS also has been proposed as an explanation of the excessive edema in the neck region of Turner syndrome fetuses.\textsuperscript{50} The aplasia was confirmed by a morphological study investigating the jugular lymphatic system of Turner fetuses.\textsuperscript{47} The authors concluded that nuchal edema in trisomy 21 and Turner syndrome fetuses is most probably caused by different mechanisms with a disturbance in endothelial differentiation of the lymphatic system in trisomy 21 fetuses and a total lack of endothelial differentiation towards a JLS in Turner syndrome fetuses.\textsuperscript{47} The bilateral cavities found in the posterior neck of Turner syndrome fetuses are most likely large nuchal cysts which are formed by accumulation of fluid in the intercellular spaces of the connective tissue.\textsuperscript{47} These cavities are comparable to the nuchal cysts seen in human fetuses and trisomy 16 mouse embryos with nuchal edema.\textsuperscript{54,55}

In summary, an abnormal or delayed lymphatic development seems to be the best explanation for increased NT. It can explain the local and temporary character of increased NT as it appears when the jugular lymphatic system undergoes its finalization. An altered endothelial differentiation of the distended jugular lymphatic system has been demonstrated. This provides an interesting link with cardiac abnormalities as endothelial development play an important role in cardiovascular development.
V. Other theories

Fetal infection

Parvovirus B19 is the only infection that is associated with increased NT. In these cases, the increased NT has been attributed to cardiac failure and fetal anemia due to a suppression of the haematopoiesis.

TTTS

It has been suggested that in monochorionic twins increased NT may be an early manifestation of the twin–twin transfusion syndrome (TTTS). It was hypothesized that the mechanism for increased NT as an early manifestation of TTTS is likely to be cardiac dysfunction due to hypervolaemic congestion in the recipient twin. According to this hypothesis, there are many bidirectional arteriovenous connections in all monochorionic twins in early pregnancy but with advancing gestation there is progressive spontaneous closure or disruption of these anastomoses. Clinical features of TTTS occur when the arteriovenous anastomoses are asymmetric so there is a net flow of blood in favor of one of the fetuses and at the expense of the co-twin.

AIM AND OUTLINE OF THE THESIS

An altered or delayed lymphatic development appears to be the best explanation for the pathophysiology of increased NT. It can explain both the local and temporary character as the jugular lymphatic system undergoes a finalization of its development at the time the NT appears. The aim of this thesis was to get more insight in the pathophysiology of increased NT. Therefore, lymphatic development in fetuses with normal and increased NT was studied using ultrasonography and immunohistochemistry.

The enlarged JLS visualised with ultrasound in fetuses with increased NT, indicate a disturbed or delayed lymphatic development. However, not much is known about the appearance of JLS on ultrasound examination in fetuses with normal NT. Chapter 2 describes the visibility and size of JLS in relation to the size of NT in fetuses with normal NT. Altered haemodynamics, indicated by altered ductus venosus Doppler flow velocities, have been suggested to play a role in the development of increased NT. In chapter 3 we investigate if there is a relation between the jugular lymphatic development and haemodynamics in the fetal neck, heart and ductus venosus of fetuses with increased NT. In chapter 4 we evaluate if ductus venosus Doppler flow alterations in fetuses with increased NT can be explained by haemodynamic changes due to a certain type of cardiac defect.

Previous research demonstrated an aberrant endothelial differentiation of the JLS in aneuploid fetuses with nuchal edema. In chapter 5 we further assess the endothelial differentiation of aneuploid fetuses with nuchal edema and euploid control fetuses using immunohistochemistry. In chapter 6 the jugular lymphatic system of euploid fetuses with nuchal edema, including
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fetuses with Noonan syndrome is described. Noonan syndrome, which is a relatively common developmental disorder associated with increased NT, is characterized by short stature, congenital heart defects, cardiac hypertrophy, a varying degree of intellectual deficit and distinctive facial characteristics. In 60-70% of the cases, a gene mutation can be demonstrated. Chapter 7 presents three cases of Noonan syndrome showing increased NT with ultrasound examination. This led to analysis of Noonan syndrome genes and as a result to the prenatal diagnosis of Noonan syndrome.

In monochorionic twins, increased NT is suggested to be a manifestation of an early form of TTTS. Chapter 8 describes discordance in NT measurements in monochorionic twin pregnancies as predictor of TTTS. Chapter 9 provides a general discussion and future perspectives on the pathophysiology of increased NT.
REFERENCES

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