Chapter 10

Summary
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The nuchal translucency (NT) is a small translucent area in the neck region of the first-trimester fetus, which normally resolves after 14 weeks gestation. Increased NT represents nuchal edema and is associated with aneuploidy. The NT measurement between 10 and 14 weeks gestation has been established, together with maternal age and serum biochemistry, as a sensitive, accurate and effective method of first-trimester screening for chromosomal abnormalities. The most frequently encountered aneuploidy is trisomy 21, followed by trisomy 18, trisomy 13 and monosomy X. A fetus with nuchal edema and a normal karyotype is still at risk of a wide spectrum of anomalies, such as structural anomalies, dysplasias, and several genetic disorders. Also, some fetuses with an increased NT present with a normal outcome. The cause for increased NT, and a link with the associated findings, remains elusive.

Recently, an abnormal lymphatic development is suggested as cause for nuchal edema because of the concomitant enlargement of the jugular lymph sacs in trisomy 16 mouse embryos with nuchal edema. Trisomy 16 mice represent a model for human trisomy 21. The lymph system develops in the neck by budding from the cardinal veins and formation of the jugular sacs. At 10 weeks gestation the jugular sacs reorganize into lymph nodes. The development completes by the ingrowth of the thoracic duct into the left jugular sac, which thereby connects the sac to the lower trunk and extremities. Subsequently, the connection of the thoracic duct into the internal jugular vein is the main site of lymphatic drainage into the system circulation. This thesis deals with the pathophysiology of increased NT and focuses on the association between jugular lymphatic development and nuchal edema.

Chapter 1 contains a brief introduction on the nuchal translucency and describes the outline of the thesis.

In chapter 2 a review of the different theories on increased nuchal translucency is provided. The variety of hypotheses can be divided in three main categories. First of all, cardiac failure is suggested because of the high incidence of cardiovascular anomalies and ductus venosus flow alterations in fetuses with nuchal edema. Secondly, various kinds of extracellular matrix alterations have been observed in the nuchal skin of aneuploid fetuses, which are suggested to cause fluid accumulation. Finally, an abnormal lymphatic development is demonstrated in fetuses with nuchal edema. We conclude that many hypotheses on NT enlargement are based on associations. Both cardiac failure and extracellular matrix alterations do not explain the regional and temporary character of nuchal edema. Nevertheless, a delayed organization and connection of the jugular sacs to the venous circulation might explain these characteristics.

In chapter 3 the presence of enlarged jugular lymph sacs was prospectively investigated in fetuses with normal and increased NT using transvaginal ultrasound. We found that an enlarged NT was significantly associated with jugular lymphatic distension on first-trimester ultrasound. Under normal circumstances the jugular sacs are not visible on ultrasound. In 22 of 26 (85%) fetuses with increased NT enlarged jugular sacs were detectable, whereas in 2 of 137 (1.5%) fetuses with normal NT. Distended jugular lymph sacs were present in fetuses...
with increased NT regardless of fetal karyotype. Also, a larger NT was associated with a higher probability of distended jugular sacs. An association between distended lymph sacs and nuchal edema, however, does not necessarily implicate a causal relation. Therefore, in chapter 4 a longitudinal analysis was performed in fetuses with nuchal edema to investigate the development of NT thickness and jugular sac volume with advancing gestation. In this study 74 fetuses with a NT >95th percentile were weekly examined by ultrasound between 11 and 17 weeks of gestational age. The volume of jugular sacs and gestational age showed a quadratic relation, which differed between euploid (n=40) and aneuploid (n=34) fetuses. The maximum volumes of the sacs were larger in fetuses with aneuploidy compared to euploidy and the sacs were longer present. Furthermore, the development of jugular lymph sac volume and increased NT were related, whereby an increase of the NT preceded enlargement of the sac. In each fetus a progressive increase in jugular sac volume was followed by subsequent decrease, with advancing gestation. However, the gestational age at the maximum size of the sacs differed between fetuses, indicating a fetus-specific pattern. A similar pattern was observed for NT development, which is in agreement with former studies. Postmortem examination confirmed distension of the JLS in all cases. Thus, we conclude that increased NT is related to a disturbed lymphatic development. Also, aneuploid fetuses seem to have a more severe disturbance of lymphatic development.

The distension of the jugular lymph sacs was found to resolve after the first-trimester, just as already is acknowledged for increased NT. However, in chapter 5 a persistent form of jugular distension and nuchal edema is described in two fetuses, which were diagnosed with Noonan syndrome after birth. The persistence of the jugular distension indicates a more severe lymphatic disturbance in the spectrum of anomalies associated with nuchal edema. In addition, both fetuses were diagnosed with a mild pulmonary stenosis, without any sign of cardiac failure, after birth.

Chapter 6 focuses on the developmental background of nuchal edema and associated lymphatic and cardiovascular anomalies. The jugular lymph sac has a close relation to the surrounding nerves and vascular structures. Nerves and endothelium have a mutual influence and share many genes in development. We studied the morphological correlation between neurogenesis and vasculogenesis in the neck, heart, and ductus venosus region of wild type and trisomy 16 mice embryos, using an antibody against Neural Cell Adhesion Molecule (NCAM). The trisomy 16 mice showed an altered arrangement of cranial nerves IX, X and XI (glossopharyngeal, vagal and accessory nerves) which are positioned between the carotid artery, jugular vein and enlarged lymphatic sac. This can be attributed to a mechanical effect of the expansion of the sac. The vagal nerve was significantly smaller, as compared to wild type embryos. NCAM was over expressed in both neuronal and cardiovascular structures in trisomy 16 mice, being particularly prominent in the 4th and 6th pharyngeal arch arteries, and the ductus venosus. In the 4th and 6th pharyngeal arch arteries, NCAM over expression was located to the part of the vessel wall that is closely related to the vagal nerve. In case of 4th pharyngeal arch artery abnormalities NCAM expression, on the other hand, was
reduced. Abnormalities of the pharyngeal arch arteries and subsequent abnormal aortic arch remodeling result in outflow tract related cardiac anomalies. Thus, the interaction between neurogenesis and vasculogenesis is disturbed in the trisomy 16 mouse model and links jugular distension, cardiovascular anomalies and ductus venosus flow alterations in human fetuses with increased nuchal translucency. This indicates a disturbed migration of neural crest cells in this mouse model.

In chapter 7 the jugular lymphatic development was investigated by postmortem examination in human aneuploid fetuses with nuchal edema and euploid controls, using different lymphatic endothelial and blood vascular endothelial markers. The jugular lymphatic endothelial differentiation was also studied in the trisomy 16 mouse model. We found a disturbed venous-lymphatic phenotype in aneuploid human fetuses and mouse embryos with enlarged jugular sacs and nuchal edema. This is indicated by an absent or diminished expression of the lymphatic markers Prox-1 and podoplanin in the enlarged jugular sac. Additionally, the enlarged jugular lymphatic sacs showed blood vessel characteristics, including increased NP-1 and VEGF-A expression. The lumen contained blood cells and smooth muscle cells lined the wall.

Therefore, a loss of lymphatic identity seems to be the underlying cause for clinical NE. Also, the abnormal endothelial differentiation provides a link to the cardiovascular anomalies associated with NE.

Monosomy X or Turner syndrome presents with a massively increased nuchal edema. This is referred to as cystic hygroma. There is an ongoing debate whether cystic hygroma must be seen as a severe nuchal edema or represents a different entity. In chapter 8 the morphology of the jugular lymph system was compared between a monosomy X fetus with a cystic hygroma, a fetus with trisomy 21 and nuchal edema and an euploid control fetus of similar gestational age.

The trisomy 21 fetus had, in contradiction of the control fetus, numerous dilated lymphatic vessels in the edematous nuchal skin. The jugular lymphatic sacs of the trisomy 21 fetus were distended and showed a diminished expression of lymphatic markers Prox-1 and podoplanin, combined with an upregulation of blood vessel markers, in contrast to the euploid fetus. The nuchal edema contained a small cyst lined by mesenchyme. The Turner fetus showed two large subcutaneous cavities in the posterior neck region, of which the cellular lining stained negative for all lymphatic and other endothelial markers. These cavities were lined by mesenchyme and are therefore considered nuchal cysts. This finding is remarkable as in several reports on Turner syndrome these cavities are assumed to present the jugular sacs. In the skin only scanty and small subcutaneous lymphatic vessels were visible and there were no jugular lymphatic sacs, adjacent to the jugular vein. Our data indicate that nuchal edema in trisomy 21 and Turner syndrome are similar morphological entities with a different size but are caused by different mechanisms. The trisomy 21 fetus showed a disturbed lymphatic endothelium differentiation resulting in an abnormal phenotype, whereas the Turner fetus showed a lack of endothelial differentiation towards jugular sacs. Both disturbances can be related to Prox-1, which is the master control gene in lymphangiogenesis.

This thesis ends with a general conclusion and future prospects in chapter 9.
First of all, these thesis shows a strong relation between jugular lymphatic distension and increased NT. Secondly, we demonstrate an abnormal venous-lymphatic phenotype in aneuploid fetuses with nuchal edema. Increased NT is associated with a broad spectrum of genetic, structural and other anomalies, with a variable extent of severity. Therefore, we conclude that there is not one single cause but that several genetic origins and epigenetic influences, linked to a common developmental process, lead to nuchal edema. This thesis identifies endothelial differentiation as the most promising candidate. A disturbed or delayed endothelial differentiation is able to cause jugular lymphatic distention and subsequent edema or result in cardiovascular anomalies as the severe end of the spectrum. The initiation of a disturbed lymphatic or blood vascular endothelial development can be either environmental or genetic. A disturbed migration of neural crest cells is suggested as etiologic factor in the lymphatic and cardiovascular anomalies in fetuses with nuchal edema, based on the findings in the trisomy 16 mice embryos. This should be further explored in the human model. In general, further investigation of the endothelial differentiation processes in fetuses with nuchal edema is warranted. Also, morphological analysis of the human ductus venosus should be performed.