Novel diagnostic and therapeutic targets in Marfan syndrome

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Marfan syndrome is a pleiotropic heritable disorder of the connective tissue. Marfan syndrome is caused by mutations in FBN1 gene. Main problem in Marfan patients is a progressive aortic root dilatation which leads to a sudden death from dissection at young age if untreated. **Chapter 1** introduces the main clinical problems and therapeutic options in Marfan patients and ends with the outline of this thesis. In recent years our understanding of Marfan syndrome pathogenesis shifted from structural weakness of the connective tissue to functional role of Transforming Growth Factor (TGF)-β. Losartan, an Angiotensine II receptor 1 blocker, a drug widely used for treatment of hypertension, emerged as a potential therapy for its TGF-β antagonistic properties and proved to have positive effects on the aortic disease in the Marfan syndrome mouse model. We and other groups worldwide initiated clinical trials investigating the effects of losartan on the aortic disease. **Chapter 2** describes the protocol of the Dutch losartan clinical trial (COMPARE study). COMPARE study is an open-label, randomized, controlled trial with blinded end-points. Treatment with losartan will be compared with no additional treatment after 3 years of follow-up while beta-blockers therapy is continued. The primary end-point is the largest change in aortic diameter at any aortic level measured by means of MRI. Secondary end-points are change in mortality, incidence of dissection, elective aortic surgery, aortic volume, aortic stiffness and ventricular function. We will also investigate gene and protein expression change in the skin under losartan therapy.

Because of the large clinical variability, Marfan syndrome (MFS) remained a clinical diagnosis despite the emerging genetic knowledge about its causes. In order to make the diagnosis of MFS uniform and distinguish it from the similar disorders, different diagnostic criteria have been set up and revised throughout years. In 2010 a revised Ghent nosology emerged which put the aortic root dilatation central in the diagnosis of MFS. In **chapter 3** we applied the revised nosology in an established adult Marfan population to define practical repercussions of novel criteria for clinical practice and individual patients. Out of 180 MFS patients, in 91% (n=164) the diagnosis of MFS remained. Out of 16 patients with rejected diagnosis, four patients were diagnosed as MASS phenotype, three as ectopia lentis syndrome and in nine patients no alternative diagnosis was established. In 13 patients the diagnosis was rejected
because the Z-score of the aortic root was <2, although the aortic diameter was larger than 40 mm in six of them. In three other patients the diagnosis of MFS was rejected because dural ectasia was given less weight in the revised nosology. Following the revised Marfan nosology, the diagnosis of MFS was rejected in 9% of patients, mostly due to absence of aortic root dilatation defined as Z-score ≥2. Currently used Z-scores seem to underestimate aortic root dilatation, especially in patients with large Body Surface Area. In addition, other parameters such as the shape of the aortic root and the relation of its diameter to surrounding structures might also indicate the aortic root disease. We concluded that a revision of the criteria for the aortic root involvement may be indicated.

As fibrillin-1 is a component of the extracellular matrix of the myocardium, mutations in \textit{FBN1} may cause impairment of ventricular function in Marfan patients. Furthermore, aortic elasticity is decreased in these patients, which might also impair ventricular function. In \textbf{chapter 4} we assessed biventricular function and the influence of aortic elasticity in patients with Marfan syndrome by means of cardiac magnetic resonance imaging. In 144 Marfan patients without significant valvular dysfunction, previous cardiac surgery, or previous aortic surgery, biventricular function and aortic elasticity were measured. We found that when compared to healthy controls, both left and right ventricular ejection fraction of Marfan patients were impaired (p< 0.005) and were strongly correlated (r=0.7, p<0.001). There was no correlation between aortic elasticity left ventricular function. These findings suggest an intrinsic myocardial dysfunction in patients with MFS.

Aortic diameters are a standard parameter of the aortic disease and are used in a daily clinical practice. However, aortic diameters seem to reflect regional expansion of the aortic wall. In \textbf{chapter 5} we measured total aortic volume in Marfan patients and explored its feasibility and reproducibility as a novel tool to assess the total aortic expansion. Gadolinium enhanced 3D MRI of the aorta was performed in 62 Marfan patients without previous aortic dissection. Mean aortic volume of 62 Marfan patients at baseline was 233 ml (SD=65 ml). Intra-observer difference was 4.63 ml (ICC= 0.996; SD=4.83). Inter-observer
difference was 0.06 ml (ICC=0.979; SD=11.96). Mean aortic volume increased from 259± 62.5 ml to 282 ±74.7 ml (Cohen’s d 0.3) and mean aortic diameter increased from 24.93 ± 2.76 mm to 25.34 ± 2.91 mm (Cohen’s d 0.1) in 15 patients. The sensitivity of aortic expansion was significantly higher using aortic volume as compared to aortic diameters (effect size 0.3 and 0.1 respectively, p<0.005). This might be of particular importance in clinical trials as it would reduce the sample size and the follow-up time needed to observe the effect of the studied drug.

In Part II of this thesis we focused on the molecular aspects of Marfan syndrome. In chapter 6 we investigated the global gene expression in skin, as a model of the connective tissue. We showed that both TGF-β and inflammation are up-regulated in patients with Marfan syndrome. We analyzed transcriptome-wide gene expression in 55 Marfan patients using Affymetrix Human Exon 1.0 ST Array and levels of TGF-β and various cytokines in their plasma. Increased plasma levels of TGF-β were found especially in MFS patients with aortic root dilatation (124 pg/ml), when compared to Marfan patients with normal aorta (10 pg/ml; p=8x10-6). Interestingly, our microarray data show that increased expression of inflammatory genes was associated with major clinical features within the Marfan patients group; namely severity of the aortic root dilatation (HLA-DRB1 and HLA-DRB5 genes), ocular lens dislocation (RAET1L, CCL19 and HLA-DQB2) and specific skeletal features (HLA-DRB1, HLA-DRB5, GZMK). Patients with progressive aortic disease had higher levels of Macrophage Colony Stimulating Factor (M-CSF) in blood. When comparing Marfan aortic root vessel wall with non-Marfan aortic root, increased numbers of CD4+ T-cells were found in the media (p=0.02) and increased number of CD8+ Tcells (p=0.003) in the adventitia of the Marfan patients. Therefore, our results imply a modifying role of inflammation in Marfan syndrome. Inflammation might be a novel therapeutic target in these patients.

Inspired by these findings, in chapter 7 we performed a genetic association study in order to investigate whether genetic variability in inflammation related genes could explain the variation in aortic disease severity in 170 MFS patients. Aortic disease severity was defined as aortic root dilatation rate over a
period of up-to 15 years. Analysis of 15169 SNPs in 943 additional inflammatory genes revealed a significant association of aortic disease severity with a SNP in the Sialic Acid Binding Ig-like Lectin 7 (SIGLEC7) gene (p=3.5x10-7). Furthermore, a trail of SNPs was observed in interleukine 16 (IL16) gene (p=7x10-5). Finally, these associations were replicated in a cohort of patients with ruptured intracranial aneurysms using the diameter of the aneurysm at the time of rupture as a proxy for the disease severity (p=0.03 and p=0.05 respectively). The SIGLEC7 gene controls innate immune response to tissue damage, while the IL16 gene is a chemoattractant of various pro-inflammatory cells and induces expression of HLA-DR genes in monocytes. We concluded that genetic variability in inflammation-related loci may modify the aortic disease severity in MFS, and possibly in other vascular diseases.

In recent years TGF-β emerged as potential prognostic and therapeutic biomarker of the aortic disease in Marfan syndrome. In addition, we assumed that patients response to losartan might also be variable, as most of the disease features in Marfan syndrome are clinically highly variable. In chapter 8 we investigated the correlation of TGF-β with the aortic phenotype and the molecular response to losartan in a group of 99 Marfan patients. Marfan patients had higher TGF-β levels than healthy controls (p=0.002). TGF-β levels correlated with larger aortic root diameter and age (p=0.05 and 0.02). Losartan reduced TGF-β levels in only 15 (36%) of 42 treated Marfan patients (responders) to levels observed in healthy controls (p=0.31). Other 27 (64%) of treated MFS patients showed no change or an increase in plasma TGF-β (non-responders). Responders had higher baseline TGF-β levels (p=0.05), more severe aortic disease and distinct FBN1 genotype as compared to non-responders. Baseline TGF-β levels and response were independent of additional beta-blocker therapy. Our results indicate that only one-third of the Marfan patients may respond to losartan if TGF-β proves to be a good therapeutic marker of its clinical effects.

Marfan syndrome is a chronic disorder which leads to many health impairments in young age. Quality of life is a novel parameter of patients well-being and it is often independent of the disease severity and
therapy suggesting it is an intrinsic feature. In chapter 9 we explored the quality of life in 121nMarfan patients and its genetic basis. We found that patients’ physical QoL was impaired and weakly related with age and scoliosis while mental quality of life was normal. To explain a largely lacking correlation between disease severity and QoL, we related genome wide gene expression to QoL. Patients with lower mental quality of life had high expression levels of CXCL9 and CXCL11 cytokine genes (p=0.001; p=0.002); similarly, patients with low vitality scores had high expression levels of CXCL9, CXCL11 and IFNA6 cytokine genes (p=0.02; p=0.02; p=0.04), independent of patient characteristics. Subsequently, we associated 484 SNPs in cytokine genes to QoL. Mental quality of life was associated with a SNP-cluster in the IL4R gene (strongest association p=0.0017). Although overall mental QoL was normal, >10% of patients had low scores for MCS and vitality. Post-hoc analysis of several related systemic inflammatory mediators showed that patients with lowest MCS and vitality scores had high levels of CCL11 cytokine (p=0.03; p=0.04). We concluded that the presence of scoliosis and activated cytokine pathway lead to impairments of Marfa patients quality of life. This is line with our previous findings over an important role of inflammation in the Marfan disease pathogenesis.

This thesis ends with a discussion of the present findings and the current literature in the light of the aortic disease pathogenesis in chapter 10.