Microfibrils of the extracellular matrix. Microfibrils regulate the activity of transforming growth factor-β (TGF-β), which is associated with larger aortic diameters [5], profibrotic processes in heart failure and are involved in myocardial remodeling processes [6]. Current treatment focuses on aortic dilation as a mortality-related end-point [7,8]. B-type natriuretic peptide (BNP) is a cardiac neurohormone synthesized mainly by ventricular myocytes as a nonspecific response to wall stress [9]. Brain natriuretic peptide (BNP) opposes TGF-β-regulated gene expression related to fibrosis and myofibroblast conversion [10]. The N-terminal prohormone of BNP (NT-proBNP) is used as a diagnostic marker for cardiac insufficiency [11], ventricular dysfunction [12,13], and aortic dissection [14]. NT-proBNP levels correlate positively with age [15], sex, and negatively with body mass index (BMI) and can be used to rule out heart failure with preserved or reduced ejection fraction (HFP EF, HFrEF) with similar accuracy [16].

The aim of the present study was to evaluate left ventricular diastolic function, type of cardio-vascular hypertrophy and NT-proBNP levels in out-patients with MFS and a control group with similar clinical manifestations referred for evaluation of suspected MFS in whom the diagnosis was ruled out. We hypothesized that subclinical diastolic dysfunction in...
patients with preserved ejection fraction, left ventricular hypertrophy and elevated NT-proBNP levels are more frequent in patients with MFS and investigated the impacting factor on NT-proBNP levels in these patients.

2. Methods

2.1. Study design and patient population

The study is a monocentric consecutive cohort study of 863 patients seen in our Marfan clinic between 1/2010 and 7/2015. The diagnosis of Marfan syndrome was based on the revised Ghent nosology [17] including molecular genetic analysis. Clinical assessment according to the Ghent criteria was performed in all patients. All patients were routinely examined in our specialized multidisciplinary Marfan clinic. Patients that did not fulfill the Ghent criteria of MFS were used as controls.

Exclusion criteria were history of cardiac surgery or aortic dissection at any time, mitral or aortic regurgitation, Loeys-Dietz-Syndrome, no definite diagnosis, and incomplete dataset (Fig. 1). No patient in this cohort was diagnosed with atrial fibrillation.

2.2. Clinical examination

History, physical examination, genetic testing, laboratory tests, orthopedic and ophthalmologic counseling and cardiovascular imaging were performed according to current recommendations. Blood creatinine, C-reactive protein and NT-proBNP levels were collected as a part of routine laboratory examination. Children were defined as patients with age < 18 years.

Two-dimensional, pulsed, and color-Doppler and color-tissue Doppler echocardiograms were acquired using phased array probes on a Vivid 7 Vingmed General Electric Ultrasound scanner (GE Vingmed Ultrasound Horten Norway) following a standardized protocol. M-mode and two dimensional recordings just beneath the mitral leaflet tips in the long axis for at least 3 beats were used for linear measurements. Left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), thickness of the posterior wall (PWD) and the septum (IVD) were measured and fractional shortening (FS), left ventricular end-diastolic volume (LVEDV), and ejection fraction (EF) according to the Teichholz’ equation [18], relative wall thickness (RWT) and LV mass (cube formula) were calculated according to recent recommendations on chamber quantification [19]. LV-geometry was defined according to the above recommendations. Left atrial diameter, aortic root diameters, early diastolic mitral flow velocity (E) and the inflow velocity during atrial contraction (A), as well as early relaxation velocity in tissue Doppler (e’, as mean of septal and lateral e’) were measured, and the E/A and E/e’ ratios were calculated. Z-scores were calculated for aortic diameter [20]. Aortic or mitral regurgitation and mitral valve prolapse were graded according to recommendations for assessment of valvular regurgitation [21].

2.3. NT-proBNP measurement

Venous blood was collected by peripheral venous puncture as part of the routine clinical evaluation in an ammonium-heparin monovette (Sarstedt, Nümbrecht, Germany). Plasma was separated by centrifugation at 4000 rpm (2700 × g) at 4 °C. Aliquots were stored at −80 °C and thawed immediately prior to NT-proBNP determination. Measurements were performed with a non-competitive sandwich electrochemiluminescent immunoassay (ECLIJA) on an Elecsys Modular E 170 platform (Roche Diagnostics, Mannheim, Germany).

2.4. Statistical analysis

SPSS® Statistics version 23, (IBM® corporation) was used. Continuous data are given as mean ± standard deviation. Categorical data are presented as percentages and analyzed by cross-table analysis. Since the NT-proBNP data were not normally distributed and cover a large range, a non-parametric test (Kolmogorov–Smirnov) and logarithmic transformation were used as appropriate. Significance level was 0.05. Binary cluster analysis was used to identify morphological and functional cardiac features that are characteristic for group differences. Univariate linear regression with single covariates was used to pre-select cofactors and covariates for linear modeling by multiple covariate analysis of variance (MANCOVA) using stepwise forward and backward selection. Medical treatment was excluded from the multivariate analysis in order to avoid bias, as any association could be caused by diagnosis-associated treatment strategy.

2.5. Ethical standards

This study complies with the requirements of our institutional Ethics Committee Review Board and is in compliance with the Helsinki standards of human medical studies. All patients gave informed consent.

3. Results

For this study we recruited 863 patients seen in our Marfan outpatient clinic. Because NT-proBNP levels are known to be raised after surgery or aortic dissection [14,22], 238 patients were excluded because they had had cardiac-vascular surgery at any time before. Four additional who had experienced an aortic dissection treated conservatively, 17 patients

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**Fig. 1.** Patient exclusion flow-chart. We analyzed 863 individual patients from 1/2010 through 7/2015. We excluded 238 patients because they had had cardiac surgery at some time in the past, and four patients that had experienced a type-B aortic dissection which was treated conservatively. Any patient with more than mild aortic or mitral regurgitation was excluded as well, as were patients who were diagnosed with Loeys-Dietz-Syndrome. Seventeen patients had incomplete datasets. The remaining 556 patients consisted of 217 with MFS, and 339 controls.
There were more dilated (24% vs. 14%, p < 0.001) and hypertrophied (35% vs. 18%, p < 0.001) ventricles in patients with MFS, however. The groups did not differ with respect to type of hypertrophic remodeling comprising about 30% excised cases in both groups. Impaired ejection fraction was found in 21 patients with MFS and 19 controls.

In binary two-step cluster analysis larger aortic diameter (38 ± 6 mm vs. 32 ± 6 mm, p < 0.001), and reduced early relaxation in tissue with diagnosis of Loey-Dietz-Syndrome, one patient in whom definite diagnosis could not be established, 13 with more than mild aortic regurgitation, 18 who had a more than mild degree of mitral regurgitation, and 16 patients in whom datasets were incomplete, were excluded as well. We investigated NT-proBNP levels and echocardiographic diastolic function parameters in 217 (110 females) patients with MFS. As a control group we investigated 339 (154 females) patients seen for suspected MFS in whom the diagnosis was subsequently ruled out (Table 1).

There were no significant group differences regarding age, height, BMI, blood pressure and heart rate. Weight was slightly but significantly higher (p = 0.023) in patients with MFS.

There were about 20% children with MFS and 17% among controls. More patients were medically treated in the MFS group (50% vs. 26%, p < 0.001). Especially treatment with angiotensin II receptor blockers (16% vs. 5%, p < 0.001) and beta-blockers (18% vs. 5%, p < 0.001) were more frequent in patients with MFS. Treatment with beta-blockers was associated with significantly increased NT-proBNP levels in both groups (p < 0.001). Mitral valve prolapse (80% vs. 44%, p < 0.001), mild mitral regurgitation (11% vs. 4%, p < 0.001) and mild aortic regurgitation (20% vs. 7%, p < 0.001) were found more often in patients with MFS.
Doppler (e’) (11.5 ± 3.3 cm/s vs. 14.6 ± 3.4 cm/s, p < 0.001) turned out to be the best classifying variables.

3.3. Analysis of cofactors and covariates predicting NT-proBNP by linear regression modeling

For linear modeling a logarithmic transformation of NT-proBNP was used.

Age (p < 0.001), gender (p < 0.001), height (p < 0.001), BSA (p < 0.001), CRP (p = 0.001), diagnosis of Marfan syndrome (p = 0.001), A-wave (p < 0.001), E/A ratio (p = 0.017), E/e’ ratio (p < 0.001), tissue Doppler measurements of septal e’ (p < 0.001) and lateral e’ (p = 0.014) and its calculated average e’ mean (p = 0.001), aortic regurgitation (p = 0.003), mitral regurgitation (p < 0.001), mitral valve prolapse (p < 0.028), aortic z-score (p = 0.002), indexed end-diastolic volume (iEDV, p = 0.032), LV hypertrophy (p = 0.003) and concentric type of hypertrophy (p = 0.039) but not LV linear dimensions or ejection fraction or creatinine were significant covariates or cofactors in predicting NT-proBNP by univariate linear analysis (see Table 2). The paradoxically negative correlation of height with NT-proBNP in the total sample is explained by hidden effects of gender and inclusion of children as demonstrated in Fig. 2. The interaction of gender and age on e’ is illustrated in Fig. 3.

In a first step of multiple parameter univariate ANCOVA (MANCOVA) linear analysis, gender (p < 0.001), age (p < 0.001), height (p = 0.001), and diagnosis of MFS (p = 0.001) were found to be significant covariates and cofactors of NT-proBNP (F-value of model 52, R² = 0.27). The diagnosis of MFS was a significant cofactor for NT-proBNP levels, as was e’, which can be regarded as a good indicator of diastolic function. MFS patients had significantly lower e’ values as compared to controls. Aortic z-score was significantly larger in the MFS group. Therefore, e’ or aortic diameter z-score were potential confounders. However, the diagnosis of MFS remained a significant cofactor even after the aortic z-score was introduced into the model, suggesting that aortic z-score has an influence on NT-proBNP levels indepently of the diagnosis of MFS. Finally in MANCOVA model adjusted for demographic parameters we investigated which parameters explain the effects of diagnosis MFS (F = 10.05) on NT-proBNP. We found that parameters of diastolic cardiac function as E/e’ (F = 10.92) and e’ mean (F = 11.72), iEDV (F = 7.70), and aortic diameter (F = 10.04) were significant and replaced diagnosis of MFS best. This analysis suggests that the impact of MFS on NT-proBNP is largely mediated by impaired diastolic function.

Fig. 2. Regression analysis of height. A negative regression in all patients (not shown) and adults (A) is explained by the higher NT-proBNP levels younger and therefore smaller children (B) and the higher NT-proBNP levels in females (C, D), who are smaller than males. This outweighs the effect of greater height of patients with MFS who do have higher NT-proBNP levels.
relaxation as well as aortic dilatation, and to a smaller amount by other factors such as concentric hypertrophy and mitral regurgitation.

4. Discussion

This is the largest clinical study on NT-proBNP levels and diastolic function parameters expressing a subclinical intrinsically cardiomyopathy in patients with MFS. Subclinical cardiomyopathy in patients with MFS has been found in several clinical echocardiographic [23–25] and magnetic resonance and combined imaging studies [3,6,26]. Diastolic dysfunction has been described to be impaired in MFS [27,28] but NT-proBNP measurements in this context have not been published before. In agreement with these studies we found a relatively impaired diastolic left ventricular relaxation as compared to controls. Left ventricular dilatation and hypertrophy without differences in type of hypertrophic remodeling and as expectedly aortic dilatation were more often seen in the MFS group.

Moreover, in this study we demonstrate significantly higher NT-proBNP levels in patients with MFS as compared to control patients with similar phenotype in whom MFS had been ruled out. This has not been described before. The relative elevation of NT-proBNP in MFS as opposed to controls was not explained by known demographic cofactors and covariates. The strongest predictor of NT-proBNP elevation was diastolic function followed by Z-score of aortic diameter suggesting that primary disease related cardiomyopathy in MFS manifests predominantly as diastolic relaxation impairment. Patients with valvular dysfunction with left ventricular loading were excluded from our study. Therefore, our results might be attributed to a MFS-related myocardial impairment as would be the case in primary cardiomyopathy, which in turn leads to diastolic dysfunction and dilatation of cardiac cavities and large vessels with resultant NT-proBNP elevation.

According to the Laplace law aortic wall stress is proportional to the aortic diameter. Our finding of a significant and independent correlation of Z-scores of aortic diameters and NT-proBNP therefore may be in part caused by ventricular afterload mismatch resulting from increased aortic stiffness. Thus some modulating role of the aortic pathology in MFS in the development of cardiomyopathy in patients with MFS is likely. Afterload impedance mismatch due to higher aortic stiffness may be related to decreased aortic elastic properties in young patients with MFS as compared to a control group [29]. Fibrillin-1 deficiency activates TGF-β signaling pathways, leading to elevated collagen synthesis and matrix metalloproteinase-mediated disruption of the elastic fibers in the vessel wall, thereby increasing aortic stiffness and decreasing vasoreactivity [30]. Increased levels of TGF-β have been detected in auneurysmatic aortic wall of patients with MFS [31] and circulating TGF-β has been proposed as prognostic biomarker in MFS [5,32].

Treatment with beta-blockers was associated with significantly increased NT-proBNP levels in both groups (p < 0.001) as has been described before [33,34]. Impairment of renal function has been shown to increase NT-proBNP levels [35]. No patient in our study had severe renal dysfunction. Blood creatinine levels were investigated and did not differ between groups and were not significant in linear modeling.

5. Limitations and conclusions

This is a non-randomized retrospective consecutive cohort study with several limitations. Data on potential clinical correlates of diastolic dysfunction, such as exercise intolerance, were not collected, and echocardiographic evaluation was performed using a minimal data set without sophisticated echocardiographic data on diastolic and systolic biventricular function as well as left and right atrial volumes, aortic compliance. Circulating TGF-β and matrix metalloproteinases were not measured. Nonetheless, our study has shown for the first time that circulating NT-proBNP levels are relatively increased in patients with MFS. Presumably this observation is related to the presence of subclinical diastolic cardiomyopathy in MFS, which conceivably could be related to the underlying genetic defect in MFS or could be a secondary effect of the increased afterload associated with increased aortic stiffness or both. Further research will be needed to characterize a potential clinical role of NT-proBNP measurements in the management of persons with MFS, and to better characterize the prevalence and clinical relevance of diastolic dysfunction in this patient group.
