Atrial fibrillation (AF) is an abnormal heart rhythm characterized by a rapid and irregular heartbeat. It is associated with an increased risk of stroke, cardiac failure, and mortality. The probability of AF increases with age; the prevalence in patients >80 years of age is >10% [1]. The incidence of AF is estimated to double by 2050 owing to the growing population of elderly people (14-17 Million in Europe) and one of four will develop AF [Figure 1] [2].

Because of a lack of sufficient diagnostic tools, the ability to discriminate between different AF subtypes (paroxysmal (PX), persistent (PE), and long-lasting persistent (LSP)) is currently limited [2], and the identification of patients at risk for therapy failure and disease relapse remains elusive. In the present study, we hypothesized that a matrix-assisted laser desorption/ionization mass spectrometry (MALDI-IMS) workflow is suitable for discriminating characteristic spatial histological changes, detecting different AF subtypes in humans.

Methods

Methods were performed as published before [3]. Briefly, patients were clinically classified according guidelines of the European Society of Cardiology. FFPE tissue specimens from PE, PX and LSP subtype were analysed by MALDI-IMS (Autoflex III, Bruker Daltonik, m/z 800-3300 Da and lateral resolution of 80 µm) and evaluated by multi-statistical testing, probabilistic latent semantic (PLSA) and receiver operating characteristic (ROC) analysis (SCiLS Lab).

Immunohistochemical analysis was performed to quantify alpha 1 type I collagen (COL1A1) protein expression in PX, PE, and LSP AF tissue samples. Further histopathological features of the different AF subtypes were graded according to an assessment of interstitial fibrosis (Masson’s trichrome and hematoxylin and eosin staining).

Results

The primary proteome screenings were simultaneously performed for PX, PE and LSP left atrial appendage tissue sections. In total, 423 m/z values from a mass range between m/z 800 and 3,500 were obtained by peak-picking and used to compare the myocardium of PX, PE, and LSP AF tissue section. Applied PLSA yields in discriminative peptide signatures between the AF subtypes. Tissue from long-lasting persistent (LSP) atrial fibrillation (AF) patients were characterized by a high value of C3 spatial tissue components (pSil-C3), in myocardial heart tissue. The different pathophysiological stages (PX, PE, LSP) could be characterized at the molecular level by their individual protein signatures (Figure 2).

The ROC analysis results in 208 peptides values that discriminate between PX and LSP myocardial tissue and 281 peptide values distinguished PX and PE in myocardial tissue regions (AUC > 0.7; p < 0.01). The identification of these peptide markers provides important insights into the disease mechanism as well as the progression of AF (Figure 3). We performed a corresponding bottom-up LC-MS/MS approach with adjacent tissue sections. In particular, alpha 1 type I collagen (COL1A1) was significantly higher in LSP and PE tissues but not in PX myocardial AF tissue. Level of interstitial fibrosis was correlated with CO1A1 activity detected by immunohistochemistry and histopathological assessment (Figure 4).

Conclusions

We report in this study, the level of atrial interstitial fibrosis clearly differed between the AF subtypes. Additionally, the level of interstitial fibrosis was correlated with CO1A1 expression detected by immunohistochemistry. This correlation might explain the fact that atrial fibrosis is a result of the imbalance between collagen synthesis and degradation. An accumulation of collagen deposits in the interstitium leads to a widening of the interstitium and alteration of the functional properties of the atrial tissue. The observation have to validated in large clinical cohort, which we will address in further studies. MALDI Imaging technique supports the exploration of specific proteomic characteristics at the spatial level with respect to different AF subtypes. Nevertheless, the challenge in AF therapy for the growing population of the elderly is the precise discrimination of different AF subtypes.

References